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Studies in the physiology of the onion plant

II. Inflorescence initiation and development, and other changes in the internal morphology of onion sets, as influenced by temperature and day length

BY O. V. S. HEATH AND P. B. MATHUR, *Research Institute of Plant Physiology,
Imperial College of Science and Technology, London*

(With Plate 7)

Previous papers have dealt with the effects of five factors on the external aspects of flowering, bulbing and ripening in onions grown from sets and also the production of sets from seed and their storage through the winter; the present communication is concerned with changes in the internal morphology of onion sets and plants from the same experiments. The previously recorded effects on flowering of high and low temperatures are confirmed and further elucidated by data from dissections, which show the changes occurring at the growing point. Thus high temperature throughout the first season's growth from seed to set exerted an after-effect, completely preventing initiation of inflorescences in the following spring. This effect may, however, have been due to the smaller size of the sets produced at high temperature. High temperature for the first 8 of the 22 weeks' experimental storage period produced a similar after-effect in delaying and greatly reducing inflorescence initiation; here there was no question of a size effect being involved. High temperature throughout the 22 weeks not only completely prevented such initiation during treatment, but also practically inhibited it subsequently; when given during the latter part of storage only, it at least delayed initiation of inflorescences. High temperature during the growth from set to mature onion showed a direct effect in suppressing the emergence of inflorescences already initiated in the sets, apart from the effect of bulbing. (Bulb development, which occurs under the stimulus of long days if the temperature is not too low, also inhibits such emergence.) Inflorescences prevented from emerging, whether by high temperature or bulbing, were displaced by the axillary bud which carried on the further development of the plant. Low temperature for the first 8 weeks of storage exerted an after-effect in reducing the incidence of inflorescence initiation. When cold storage was given for the whole 22 weeks or the last 8 weeks only, initiation was prevented during treatment, but whereas in the former case subsequent initiation was greatly reduced, in the latter a rapid flush of initiation occurred on return of the sets to normal temperature. The effects of high- and low-storage temperatures on formation of new leaf initials were mainly direct; the only after-effect was a tendency for sets from treatments preventing leaf initiation to produce initials more rapidly on return to normal temperatures. (In the case of late cold storage this resembled the effect just noted for inflorescence initiation.) Sets produced at low temperature were prevented from forming leaf initials by continuous high-temperature storage and those produced at high temperature by continuous cold storage; this suggests some degree of acclimatization to low and high temperature respectively. It appears that the 1 to 2 leaf primordia formed at normal temperatures between mid-October and mid-March are initiated mainly during the latter part of that period. The number of swollen bulb scales in an onion set is nearly constant at 3, irrespective of set size. Even the unswollen leaf initials vary little in number over a considerable range of set size, and therefore the better yields given by large sets (if they do not bolt) must be due rather to large size of parts than to high number of leaf initials ready for emergence. The size effect on flowering is likewise not connected with number of leaf initials present in the sets; it *may* be a matter of the number of emerged leaves in the previous season. There appears to be a minimal total number (12-14) of leaves which must be initiated before an inflorescence can be formed.

INTRODUCTION

In the investigation of the effects upon the flowering behaviour of plants of such external factors as temperature and day length, information as to the changes occurring at the growing point is likely to be of the greatest importance, as has been demonstrated by the work of Purvis (1934) and Purvis & Gregory (1937) on the vernalization of cereals. This information can only be gained by careful dissections or sectioning, and in order to obtain statistically satis-

factory estimates of such quantities as the incidence of flower initiation, or a change in number of leaf primordia, it is generally necessary to deal with very large numbers of plants. Thus, in the present paper, the data from a total of some 3200 dissections* are involved, but nevertheless the sampling errors are in some cases unduly high. The method is laborious,

* Of these, the first 1200 were carried out by Miss E. Bruck, to whom grateful acknowledgement is made, and the rest by P. B. Mathur.

in the pointed structure which is the future leaf blade, and since this also grows centripetally and overhangs the axis, the pore is left on the opposite side. Each new leaf initial arises within the surrounding sheath of the preceding one, with the tip of its embryo blade just below or just in the pore through which it may ultimately be expected to emerge (Pl. 7, fig. 2). Up to the onset of bulbing new leaves emerge about once a fortnight, and there are generally three unemerged leaf initials at the growing point (Heath, 1943*a*, p. 212). When the plant begins to form a bulb, leaf emergence ceases immediately or soon (Heath, 1943*a*, p. 212; 1943*b*, pp. 314, 318), and is not resumed until after a period of dormancy; the production of leaf initials continues, apparently very rapidly, until there are about nine unemerged initials, or 'scales' (Pl. 7, fig. 1). The process of bulbing consists in the swelling of the sheathing bases of one or more of the innermost of the emerged leaves and also the sheaths of three (occasionally two or four) of the outermost unemerged leaf initials. The former are denoted in this paper as 'swollen leaves', the latter as 'swollen scales' and unemerged leaf initials in general are termed 'scales'. In small sets the swollen leaves are often absent. In due course the bulb ripens off and enters upon a dormant period (Heath, 1943*a*, p. 213; 1943*b*, p. 312). With ripening, activity at the growing point almost ceases and only one or two further leaf initials are formed during storage from October to March at ordinary temperatures (see Table 5). Then, usually during March, in large sets produced and stored at ordinary temperatures, the central axis bearing the terminal growing point begins to elongate to form a scape. At the same time a spathe is formed and a lateral bud develops in the axil of the innermost scale (Pl. 7, figs. 3, 4). This stage of inflorescence development appears to be reached rapidly, but no further development occurs until after planting out (cf. Pl. 7, figs. 3, 4). Then the seven or eight *unswollen* scales begin to elongate in serial order (cf. Boswell, 1923). The three *swollen* scales show little or no growth, and never produce leaf blades but later shrivel and rot away. Under appropriate conditions the inflorescence (if present) emerges in its turn from the sheathing base of the innermost (youngest) leaf on the main axis, and any further growth is then carried on by the axillary bud. It may happen, especially in large onions, that axillary buds develop much earlier than indicated above, and thus a bulb with several growing points is formed before the storage period. This did not occur in the present experiments and has been omitted from the description of 'normal' development for the sake of simplicity.

MATERIAL AND METHODS

Sets of var. Ailsa Craig (Prize Taker Stock—Ryder) were produced at high and low temperature (*F* and *f*, see Table 1) and with five germination dates (*I* to *V*,

Table 1) (see Heath, 1943*a*, pp. 211 *et seq.*). To obtain a supply of sets of known leaf number for dissection, the leaves of large numbers of seedlings were marked during the growing season (Heath, 1943*a*, p. 212). Such 'leaf-marked' sets were heavier than the 'unmarked' sets (Heath, 1943*a*, p. 213 and Table 3, p. 214), but although they did not constitute representative samples of the lots from which they were taken they were comparable among themselves. The heaviest and lightest of the leaf-marked sets were removed, and these weight classes were the same as those discarded from the *unmarked* sets in which they constituted approximately the 5% tails of the weight distribution. This meant the removal of more large than small bulbs from the *leaf-marked* sets owing to their greater mean weights. The ranges of weight remaining after the extremes had been removed, and also the mean weights of both leaf-marked and unmarked sets after such removal, are shown in Table 3 of the first paper (Heath, 1943*a*, p. 214).

On 10–12 Oct. 1940, after grading and immediately before the beginning of the storage treatments, there was pickled for dissection a random sample of thirty leaf-marked sets from each duplicate (A or B) of each time-temperature combination (e.g. *II*/*F*). This material provided data as to the internal morphology at the beginning of storage and so gave a datum line from which the effects of storage treatment could be assessed. The over-size and under-size leaf-marked sets from certain treatment combinations (*II* and *IV* combined with *F* and *f*, Table 1) were also pickled 15–16 Nov. 1940 to ascertain the relations between scale number, leaf number and weight (p. 177).

Some data are presented (p. 177) derived from leaf-marked sets pickled at an analogous stage in an experiment in 1941–2 with var. Bedfordshire Champion (Ryder). These sets were produced under conditions similar to those for the low-temperature (*f*) sets in the present experiments. They were much smaller, however, and there was little disparity in weight between the leaf-marked and unmarked sets. After discarding the extremes (3% tails) the sets were graded into three weight classes (of almost equal frequency) within each of the two germination dates. A random sample of twenty-five leaf-marked sets was pickled from each duplicate lot of each of the weight-germination date combinations.

After the initial pickling on 10–12 Oct. 1940, the remainder of the leaf-marked sets in the Main Experiments, 1940–1, were allotted to the ten storage treatments (see Table 1) in almost equal numbers (Heath, 1943*a*, p. 214) and subjected to the same conditions as the unmarked sets. Half of them were pickled at the end of the full treatment period of 22 weeks (13–15 Mar. 1941) to give information as to the internal changes which had occurred during treatment. The rest were kept under 'common' storage conditions (*C*, see Table 1) for a further

6-7 weeks and were then also pickled (29 Apr.-1 May 1941) in order that *after-effects* of the storage treatments might be assessed. This last material also provided an approximate estimate of the condition of the unmarked sets at the time they were planted in the greenhouse experiment on 16-17 Apr. 1941 (see below). Such an estimate was, however, subject to two sources of systematic error: first, the sets for dissection were pickled about a fortnight after the planting of the greenhouse experiment, and secondly, the marked-leaf sets were on the whole larger than the unmarked sets.

alcohol after making an oblique cut from the shoulder towards the growing point to facilitate rapid entry of the fixative. The material was kept in the alcohol until required. The leaf-marked sets pickled before and after storage treatment and 6-7 weeks later were hand-sectioned after the removal of the swollen leaves and swollen scales. Only longitudinal sections cut in the plane of the leaf initials were used, and care was taken that these should be truly median (cf. Pl. 7, fig. 1). In non-median sections it is easy to miss a leaf initial and even possible to miss the early stages of inflorescence initiation. The plants pickled

TABLE 2. *Main experiments, 1940-1. Initial dissections of leaf-marked sets at beginning of storage treatments*

Mean number of scales (swollen + unswollen) on 10-12 Oct. 1940 after removal of extreme weight classes.
(For symbols see Table 1, p. 174)

	<i>f</i>					<i>F</i>					Mean
	<i>I</i>	<i>II</i>	<i>III</i>	<i>IV</i>	Mean	<i>I</i>	<i>II</i>	<i>III</i>	<i>IV</i>	<i>V</i>	
Wt. range (g.)	1.0-8.0	1.0-7.0	1.0-7.0	0.7-7.0		0.7-6.0	0.5-4.0	0.35-4.0	0.35-3.5	0.35-3.5	
No. of emerged leaves											
2 A	—	—	—	—	—	—	—	—	8.5	7.8	8.0
B	—	—	—	—		—	—	—	(8.0)	7.8	
3 A	—	—	—	—	—	—	—	8.1	8.3	8.2	8.6
B	—	—	—	—		—	—	10.0	8.4	8.3	
4 A	(9.5)	(9.3)	(9.0)	9.3	9.3	—	9.3	8.8	8.6	8.7	8.8
B	(9.5)	(10.0)	(9.0)	8.6		—	(8.5)	8.8	8.9	8.4	
5 A	9.8	9.2	9.7	8.5	9.3	8.6	9.5	8.7	(8.5)	(9.0)	9.0
B	9.5	9.5	9.5	8.7		9.0	9.0	8.9	(9.5)	—	
6 A	9.7	9.3	9.4	9.4	9.4	9.6	9.3	(9.0)	(8.0)	—	9.0
B	9.5	9.6	9.3	9.0		9.6	8.8	(9.0)	—	—	
7 A	(8.4)	(9.0)	9.2	9.4	9.1	9.8	(7.0)	—	—	—	9.8
B	(9.5)	(10.3)	9.0	(8.0)		10.3	(12.0)	—	—	—	
8 A	(9.0)	—	—	—	—	(10.5)	—	—	—	—	—
B	—	—	—	—		—	—	—	—	—	
Mean* A	9.5	9.3	9.5	9.0	—	9.4	9.3	8.6	8.4	8.1	—
B	9.5	9.6	9.3	8.7	—	9.6	9.0	9.0	8.6	8.1	—

* Weighted means allowing for varying frequencies of sets in different leaf-number classes, i.e. true means of random samples of thirty sets.

Note. Values in brackets derived from dissection of three sets or fewer.

Unmarked sets from all the treatment combinations were planted out in the field without further differential treatment, thus forming a 3-factor experiment (Heath, 1943*a*). No dissections were carried out on these plants. A greenhouse experiment involving two temperatures and two day lengths combined with certain of the earlier treatments (see Table 1) was also planted with unmarked sets (Heath, 1943*b*), and at the end of this 5-factor experiment all the surviving plants were pickled for dissection.

The method of pickling used throughout, with the exception of certain material fixed in Carnoy's fluid for microtoming, was to immerse the bulb in 50%

at the end of the greenhouse experiment were not sectioned but dissected. The sheathing leaf bases were removed one by one under a dissecting microscope and search was made for suppressed inflorescence primordia.

EXPERIMENTAL RESULTS

Initial dissections, before storage treatment

The sections of the material pickled 10-12 Oct. 1940 showed that the growing points of all sets were then in the vegetative condition (Pl. 7, fig. 2). The total number of scales (unemerged leaf initials) in each set

was counted, but the swollen scales were not recorded separately. The mean scale numbers are shown in Table 2 for each leaf-number class in every combination of temperature (*f*, *F*) and germination date (*I-V*). Since the random samples of thirty sets (e.g. from *IVfB*) covered all leaf numbers, and these occurred with varying frequency, the means are based on different numbers of sets; those shown in brackets are based on fewer than four. At the foot of the table are given the means for the complete samples of thirty sets. The weights of the actual sets dissected are not known, but valid though rather inaccurate estimates are provided by the mean weights of the larger samples of leaf-marked sets from which they were drawn. In the calculation of correlation and regression coefficients, the mean scale numbers shown in the body of Table 2 have been used with the corresponding leaf numbers and mean weights (i.e. 'between arrays'), but allowance has been made for the varying frequencies.

It is clear that mean scale number varies very little. In the *f* sets produced out of doors it is practically constant, and for a given germination date there is no evidence that it is affected by either leaf number or mean weight. Thus the total correlation of scale number and leaf number *within dates* gives $r_{sl} = -0.09$ (with $n=31$), and that of scale number and mean weight, $r_{sw} = +0.04$ ($n=31$). The partial correlations, eliminating the effects of weight or leaf number, are higher but fail to reach significance ($r_{sl \cdot w} = -0.33$, $r_{sw \cdot l} = +0.30$; $n=30$ and $P>0.05$ in each case). Leaf number and mean weight are highly correlated *within dates* ($r_{lw} = +0.79$, $n=31$, $P<0.01$) as might be expected. As *between* different germination dates, the only significant correlation, total or partial, among the three variables considered is the total correlation of scale number and leaf number ($r_{sl} = +0.95$, $n=2$, $P<0.05$). Thus the small differences in mean scale number between germination times are closely associated in a linear relation with those in mean leaf number. The regression coefficient is $+1.30$ scales per leaf, but the range for mean leaf number is only from 5.2 for *IVf* to 5.7 for *IIf* and *IIIIf*.

In the *F* sets produced under glass the range both of leaf number and scale number is greater. As in the *f* sets, leaf number and mean weight are highly correlated *within dates* ($r_{lw} = +0.91$, $n=33$, $P<0.01$). The total correlations of scale number with leaf number and with mean weight are also very significant ($r_{sl} = +0.48$, $r_{sw} = +0.55$; $n=33$ and $P<0.01$ in each case), but the partial correlations eliminating the effects of weight or leaf number are both non-significant ($r_{sl \cdot w} = -0.04$, $r_{sw \cdot l} = +0.29$; $n=32$ and $P>0.1$ in each case). Thus owing to the high correlation of the two 'independent' variates, leaf number and mean weight, their effects on scale number cannot be separated. *Between germination date means*, the three total correlations among the variables considered are all significant ($r_{sl} = +0.99$, $n=3$, $P<0.01$;

$r_{sw} = +0.89$, $n=3$, $P<0.05$; $r_{lw} = +0.90$, $n=3$, $P<0.05$). With the elimination of the leaf-number effect, however, the partial correlation of scale number and weight is found to be almost zero ($r_{sw \cdot l} = -0.08$, $n=2$). On the other hand, the partial correlation of scale number and leaf number, eliminating weight, is significant ($r_{sl \cdot w} = +0.96$, $n=2$, $P<0.05$). The corresponding partial regression coefficient is $+0.40$ scales per leaf. The range of mean leaf numbers is from 6.0 (*IF*) to 2.9 (*VF*). As in the *f* sets there is thus a close linear relation between the mean leaf number and mean scale number for different germination dates, but there is no evidence of an effect of weight apart from leaf number.

The main purpose of these initial dissections was to estimate mean scale numbers in order to detect any increases during storage treatment. In view of the unexpected constancy of these numbers, however, further information was sought by the dissection of over-weight and under-weight sets pickled 15-16 Nov. 1940. The data for under-weight sets are too scanty to tabulate, but the thirteen available sets in *IIF* and *IVF*, each weighing about 0.2 g., gave scale numbers ranging from 6 to 7. The lightest sets thus have appreciably fewer scales than those near the modal weight. The data in Table 3 for the over-weight sets are means based on ten or fewer dissections, and values from three or fewer are shown in brackets. The mean weights are for the actual sets dissected. There is no indication of a correlation between scale number and leaf number, either for *f* or *F*. Nor, among themselves, do these data indicate a correlation of scale number and weight. In conjunction with the results presented earlier, however, they show clearly the much higher scale number of the heaviest sets, and there is thus no doubt that weight and scale number are correlated. Had these over-weight or under-weight classes been included in the main dissections the effects on the mean scale numbers would have been small owing to their relatively low frequencies.

The initial dissections of leaf-marked sets from the experiment with var. *Bedfordshire Champion*, 1941-2, provided data sufficiently symmetrically distributed to be subjected to analysis of variance. Analysis on scale number was carried out separately for the two germination dates as the weight and leaf-number classes were different. Table 4 gives the means for these classes, both in respect of total scales per set and also 'swollen scales', together with the standard errors and differences required between means for statistical significance. The number of scales per set was practically the same as in the *f* sets (Table 2). In each germination date series there was a small but significant effect of set weight: heavy sets had a somewhat higher number of *total* scales than light, thus confirming the relation between weight and scale number suggested by the dissections of over-weight and under-weight *f* and *F* sets. The

TABLE 3. *Main experiments, 1940-1. Dissections of leaf-marked sets shortly after beginning of storage treatments*

Mean number of scales (swollen + unswollen) and mean weights in g. (italics) on 15-16 Nov. 1940 of sets in over-weight classes discarded from experiment

(For symbols see Table 1, p. 174)

		<i>f</i>						<i>F</i>					
		<i>II</i>		<i>IV</i>		Mean		<i>II</i>		<i>IV</i>		Mean	
Wt. class (g.)		>7.0		>7.0				>4.0		>3.5			
No. of emerged leaves													
3	A	—		—		—		—		11.8 4.1 }		11.2 4.0	
	B	—		—		—		—		10.5 3.9 }			
4	A	—		—		—		—		11.6 4.1 }		11.3 4.5	
	B	—		—		—		—		11.0 4.8 }			
5	A	11.8	7.5	(11.7)	(8.1)	12.0	7.1	11.1	5.2	11.5	6.6	11.3	5.4
	B	12.8	7.4	11.8	5.2			11.9	4.7	10.8	5.0		
6	A	12.1	8.6	11.3	8.1	11.6	8.4	11.9	5.7	—		11.8	5.6
	B	12.0	8.7	11.0	8.0			11.9	4.6	11.6	6.4		
7	A	11.9	7.9	11.0	8.4	11.6	8.1	11.5	6.5	—		12.3	5.9
	B	12.0	8.5	(11.5)	(7.8)			(13.0)	(5.3)	—			
8	A	(11.0)	(9.5)	—		11.3	10.6	11.0	6.4	—		11.5	6.9
	B	(11.5)	(11.6)	—				12.0	7.3	—			
Mean		11.9	8.7	11.4	7.6	—		11.8	5.7	11.3	5.0	—	

Note. Values in brackets derived from dissection of three sets or fewer.

TABLE 4. *Experiment with var. Bedfordshire Champion, 1941-2. Initial dissections of leaf-marked sets at beginning of storage*

Mean number of scales (total and also swollen) on 22-27 Oct. 1941 after removal of extreme weight classes (3% 'tails' of distribution)

	1st germination date			2nd germination date		
	4	5	6	3	4	5
Leaf number class:						
Mean no. of scales (total)	9.65	9.53	9.18	9.47	9.37	9.18
Mean no. of swollen scales	2.92	2.88	2.63	2.85	2.95	3.12
Weight class (g.):	0.4-0.9	0.9-1.3	1.3-2.3	0.3-0.6	0.6-0.9	0.9-2.0
Mean no. of scales (total)*	8.83	9.50	10.03	9.08	9.25	9.68
Mean no. of swollen scales*	2.78	2.77	2.88	2.90	2.97	3.05
Scales (total):						
s.e. of a single mean		0.075			0.124	
Significant diff. for <i>P</i> 0.05		0.24			0.40	
Significant diff. for <i>P</i> 0.01		0.35			0.57	
Swollen scales:						
s.e. of a single mean		0.066			—	
Significant diff. for <i>P</i> 0.05		0.21			—	
Significant diff. for <i>P</i> 0.01		0.31			—	

* Each mean based on dissection of fifty sets.

mean number of *swollen* scales, however, shows no significant variation between weight classes; the number is almost constant and nearly all the variation in total scales is accounted for by the unswollen ones which can affect the weight very little. This suggests that high weight is not caused by high scale number but by *large* swollen scales (and, in some cases, swollen leaf bases, but very few of the sets in question had these). The means in the leaf-number classes

show in each germination date series a small *reduction* in number of total scales with increasing leaf number. This reduction is, however, only significant in the first germination date series in which it is largely due to a similar significant reduction in number of swollen scales. These data therefore give no suggestion of a *positive* correlation of leaf number and scale number within germination dates; in this respect they agree with the *f* data.

*Dissections at the end of storage treatment
and 6-7 weeks later*

(a) *Increase in scale number during storage*

The method of calculation was to find for each set dissected the *difference* of the total scale number from the mean for the same leaf-number class in the corresponding lot pickled at the beginning of storage (e.g. 5-leaf, *IIIFB*, 10-12 Oct. 1940). This was done both for the material pickled 13-15 Mar. 1941 at the end of storage treatment (2-8 sets dissected per duplicate lot, e.g. *IIIFT₂B*) and for that pickled 29 Apr.-1 May 1941 (four sets dissected per duplicate

1 May 1941, +1.33 scales. Compared with the random variance this effect was highly significant ($P < 0.001$), and there is no doubt that in the particular selection of treatments given there was a real increase in mean scale number during the 6-7 weeks' period. The main effect of first season temperature (F, f) was significantly greater than the interactions in which this factor was involved ($P < 0.05$) and is therefore of general application. The means were: F , +0.96 scales; f , +1.44 scales. The germination date effect ($I-IV$) was non-significant, but that of storage temperature was significant ($P < 0.05$) when tested against its interactions and the treatment

TABLE 5. *Main experiments, 1940-1. Dissections of leaf-marked sets at end of storage treatment (13-15 Mar. 1941) and 6-7 weeks later (29 Apr.-1 May 1941)*

Change in number of scales (swollen + unswollen) since beginning of storage treatment (10-12 Oct. 1940).										
Figures in <i>italics</i> not significantly greater than zero, figures in heavy type indicate significant increase from the first to the second occasion.										
(For symbols see Table 1, p. 174)										
	High temp.							Low temp.		
	T_1	T_2	T_3	T_4	T_5	T_6	C	t_2	t_3	t_4
(a) Storage treatment means over both occasions and all levels of other factors	+1.56	+1.23	+0.55	+0.99	+1.29	+1.11	+1.61	+1.64	+0.77	+1.27
	S.E. of a single mean = 0.178									
	Significant diff. required for $P 0.05 = 0.57$									
	Significant diff. required for $P 0.01 = 0.82$									
(b) Means over all germination dates (<i>I-IV</i>)										
13-15 Mar.										
<i>f</i>	+1.79	+1.57	-0.09	+1.03	+1.80	+0.98	+1.98	+2.07	+0.69	+1.15
<i>F</i>	+1.11	+0.85	+0.40	+0.78	+0.97	+1.05	+1.14	+1.23	+0.21	+0.85
29 Apr.-1 May										
<i>f</i>	+1.95	+1.66	+1.27	+1.43	+1.46	+1.66	+1.60	+1.65	+1.16	+1.99
<i>F</i>	+1.40	+0.84	+0.62	+0.73	+0.92	+0.75	+1.71	+1.61	+1.03	+1.09
	S.E. of a single mean = 0.211									
	Significant excess over zero $P 0.05 = 0.35$									
	Significant excess over zero $P 0.01 = 0.49$									
Significant difference <i>within</i> one occasion, assuming that either of two means may significantly exceed the other:										
	Required for $P 0.05 = 0.59$									
	Required for $P 0.01 = 0.78$									
Significant difference <i>between</i> occasions, assuming that only an increase from the first to the second can be significant:										
	Required for $P 0.05 = 0.50$									
	Required for $P 0.01 = 0.71$									

lot). The random variances within the various combination treatments and for both occasions were found to be independent of the treatment means. A single comprehensive analysis of variance, including both occasions, was therefore carried out on the data for change in number of scales since the beginning of storage. For the method used in comparing average effects of single factors with 'errors' derived from their significant interactions, and of comparing low-order interactions with significant higher order interactions, see Heath (1943*a*, p. 215).

The average effect of 'occasion' (pickling at the end of storage treatment *v.* pickling 6-7 weeks later) nearly reached the 5% significance level, the means being: 13-15 Mar. 1941, +1.08 scales; 29 Apr.-

means are presented in Table 5 (a). These show the smaller increases in scale number for sets stored throughout the winter either at high (T_3) or low temperature (t_3) than at 'common storage' temperatures (C). Late heat treatment for 8 weeks (T_4) also reduced scale formation significantly, whereas early heat treatment for the same length of time (T_2) did not.

Only one of the 2-factor interactions reached significance, that between germination date and storage temperature ($I-IV$ *v.* $T-t$, $P < 0.05$). The relation was extremely complex, and since no interpretation suggests itself the means are omitted from Table 5 in order to save space. Of the 3-factor interactions, that between first season temperature, storage tem-

perature and pickling date was very significant ($P < 0.01$) and of considerable interest. The relevant means are presented in Table 5 (b). For material pickled immediately after the storage treatments (13-15 Mar. 1941) the sets produced at low temperature (f) and stored throughout at high temperature (T_3) gave no evidence of having formed any new scales during storage, whereas the sets produced at high temperature (F) showed a significant increase in this storage treatment. For the continuous cold storage treatment (t_3) the converse held, F sets giving no evidence of new scale formation but f sets showing a significant increase. These results suggest some degree of acclimatization of the F and f sets to high and low temperature respectively. Among the various storage treatments of f sets pickled on this first occasion, the mean increase for the C treatment

sets gave no evidence of any increase, and this again is similar to the effects found for inflorescence initiation. The fT_3 and Ft_3 sets, which showed no significant increase during storage treatment, considerably and significantly increased their scale numbers in this 6-7 weeks period. The ft_3 and FT_3 sets, on the other hand, gave much smaller increases which were non-significant.

The 3-factor interaction between germination date, first season temperature and pickling date was also very significant ($P < 0.01$). No interpretation is suggested and these data will not be presented or discussed.

(b) *Inflorescence initiation—effects of treatment*

The data here considered are the percentages of sets in the duplicate lots (of 2 to 8, or 4; see p. 179)

TABLE 6. *Main experiments, 1940-1. Dissections of leaf-marked sets at end of storage treatment (13-15 Mar. 1941) and 6-7 weeks later (29 Apr.-1 May 1941)*

Percentage of sets (produced at low temp., f) with inflorescence primordia

(For symbols see Table 1, p. 174)

Means over germination dates I-III

High temp.							Low temp.		
T_1	T_2	T_3	T_4	T_5	T_6	C	t_2	t_3	t_4
(a) Sets pickled 13-15 Mar. 1941 (means based on 16-29 sets each)									
34.5	—	—	9.5	9.8	25.8	33.0	18.0	—	—
(b) Sets pickled 29 Apr.-1 May 1941 (means based on 24 sets each)									
20.8	16.7	—	20.8	12.5	16.7	45.8	16.7	12.5	41.7
S.E. of a single mean						= 6.37			
Significant diff. required for $P 0.05$						= 18.5			
Significant diff. required for $P 0.01$						= 24.9			
(c) Difference, 29 Apr.-1 May minus 13-15 Mar. 1941									
-13.7	+16.7	—	+11.3	+2.7	-9.2	+12.8	-1.3	+12.5	+41.7
S.E. of a single mean						= 10.06			
Significant excess over zero						$P 0.05 = 17.1$			
Significant excess over zero						$P 0.01 = 24.9$			
Significant diff. between means						$P 0.05 = 29.2$			
Significant diff. between means						$P 0.01 = 39.4$			

was significantly above those for T_3 , T_4 , T_6 , t_3 and t_4 . All these were either continuous or late high- or low-temperature treatments; none of the early treatments gave a significant difference from C . The storage treatment differences among the F sets were on the whole similar, but only T_3 and t_3 were significantly below C . These results indicate that a considerable part of the scale formation at normal temperatures during the period mid-October to mid-March occurs in the last 8 weeks, at least in f sets. The ft_4 sets formed significantly fewer scales than the ft_2 sets during storage treatment, and a similar but even more striking difference has been found for inflorescence initiation (see below). For the 6-7 weeks' storage at normal temperatures from 13-15 Mar. to 29 Apr.-1 May the ft_4 sets showed a large and highly significant increase in scale number whereas the ft_2

which showed elongation of the main axis to form a scape (p. 175). None of the F sets dissected showed any signs of inflorescence initiation, either on 13-15 Mar. 1941 (428 sets) or 29 Apr.-1 May (324 sets); only f data are discussed therefore.

The f sets pickled 13-15 Mar. showed no elongation of the axis in storage treatments T_2 , T_3 , t_3 or t_4 (28-32 sets in each), and only two out of seventy-three dissections of sets from germination date IV showed inflorescence initiation. The data from the remaining treatments gave a mean percentage of 21.8 ± 6.1 ($P 0.05$) but without significant differences between treatments. The means are shown in Table 6 (a).

By 29 Apr.-1 May, further initiation had occurred, and all storage treatments except T_3 gave some sets with inflorescence primordia. Analysis of variance for this occasion, omitting T_3 , showed a very signi-

ficant effect of germination date ($P < 0.01$); the mean percentages were: *I*, 26.4; *II*, 20.8; *III*, 20.8; *IV*, 11.1. The *I* minus *IV* difference was very significant ($P < 0.01$), but *II* or *III* minus *IV* just failed to reach the $P = 0.05$ level. The average effect of storage treatment was also significant ($P < 0.05$). Omission of *IV* from the analysis caused the disappearance of the significant germination date effect, but the storage treatment effect was scarcely changed and the treatment means are given in Table 6 (*b*). The sets from the late cold-storage treatment t_4 , which entirely prevented initiation during storage, had by 29 Apr.–1 May practically as many inflorescence initials as those from the *C* treatment. The means for these two treatments, *C* and t_4 , were significantly above all others, which except for T_3 , not included in the analysis, did not differ significantly among themselves.

Analysis of variance was also applied to the differences 29 Apr.–1 May minus 13–15 Mar., omitting the data for T_3 and *IV*. The storage treatment effect was significant ($P < 0.05$), and the mean differences are given in Table 6 (*c*). The t_4 sets showed a large and very significant increase in inflorescence primordia ($P < 0.01$), but no other treatment showed a significant change although the increase for T_2 practically reached the $P = 0.05$ level. The difference in increase between t_4 and *C* also all but attained this level, and the t_4 mean was significantly above all the others except T_2 .

(*c*) *Inflorescence initiation—relation to number of scales and leaves*

Three considerations will be dealt with here: (1) Since the inflorescence is terminal, it would appear that there must be a certain *minimal* number of leaves formed at the growing point before an inflorescence can be initiated (Gregory, 1936), as is the case for rye and other cereals (Purvis, 1934; Purvis & Gregory, 1937). (2) There may be also, as in rye (Purvis, 1934; Purvis & Gregory, 1937), a *maximal* leaf number, above which inflorescence initiation *must* occur whatever the treatment. (3) There may be a general *tendency* for higher leaf number to encourage inflorescence initiation.

(1) *Minimal leaf number.* From the dissections of *f* sets pickled at the end of storage and 6–7 weeks later, the numbers recorded for leaves plus scales (i.e. total number of leaf primordia produced by the growing point) ranged from thirteen to twenty-two. In those treatments where some inflorescence initiation had occurred, only one out of a total of eleven sets in the '13' class had an inflorescence primordium; out of forty-four sets with 14 leaves + scales, nine had initiated inflorescences. Thus the minimal number was 14 or less. Some data obtained in 1943 by one of us (P.B.M.), working with onions of var. Ailsa Craig (Prize Taker Stock—Ryder) grown from spring-sown seed, are of interest here. Practically all

the plants flowered in the first season—conditions as different from the use of sets as could be imagined. The mean number of leaves before flowering was 13.6 and the minimum 12. A minimal number of 12–14 therefore seems most probable.

(2) *Maximal leaf number.* The highest numbers of leaves plus scales observed in these dissections were: one set with 22 and one with 21, both in the vegetative state, and thirteen sets with 20, only two of which had inflorescence initials. Clearly the maximal leaf number, if it exists, is not reached in these data. Heath & Holdsworth (1943), using plants from the Bedfordshire Champion Experiment mentioned above (p. 175), found inflorescences initiated after a total of about 34 leaves plus scales had been formed.

(3) *General tendency.* Inflorescence initiation may tend to increase with a higher number of leaves plus scales, or with one or the other separately. In view of the high positive correlation of emerged leaf number and set weight (p. 177), and the well-established effect of large set size in promoting bolting (Thompson & Smith, 1938; Heath, 1943*a*), it would be expected that in all storage treatments the sets with inflorescence initials would be those which had more emerged leaves in 1940. The data in Table 7 (*a*), for the difference in mean number of emerged leaves of sets with and without inflorescence primordia, give little indication of this for 13–15 Mar.; but for 29 Apr.–1 May, when more inflorescences had been initiated in some treatments, there is a general tendency of this kind. The irregular and scanty distribution of inflorescences renders the data in Table 7 (*a*) unsuitable for treatment by analysis of variance, and another method of testing the significance of the effect under discussion was used. For each storage treatment separately a 2×2 contingency table was drawn up, showing the frequency of sets with and without inflorescence initials in the leaf-number classes above and below the approximate median value (Table 7 (*b*)). The significance of the deviation from proportionality was found by the exact test, based on the binomial distribution, described in Fisher (1941, § 21.02). By this method, no significant relation could be established between presence of inflorescences and number of emerged leaves for the sets pickled 13–15 Mar. The most nearly significant deviations were in T_4 and T_6 (Table 7 (*b*)), and were in opposite directions. For sets pickled 29 Apr.–1 May, however, significant effects were found for *C* and T_1 (Table 7 (*b*)) such that presence of inflorescence initials was associated with high leaf number.

The same methods were applied to the relation of inflorescences to number of scales, but no significant effects were found although T_6 showed appreciable deviations in the same direction as for emerged leaves ($P = 0.244$ for the March pickling and $P = 0.067$ for that on 29 Apr.–1 May).

TABLE 7. *Main experiments, 1940-1. Dissections of leaf-marked sets at end of storage treatment (13-15 Mar. 1941) and 6-7 weeks later (29 Apr.-1 May 1941). f sets only*

(For symbols see Table 1, p. 174)

(a) Difference in mean number of emerged leaves (1940) for sets with and without inflorescence primordia. (A+B taken together.)

13-15 Mar. 1941

	T_1	T_2	T_4	T_5	T_6	C	t_2	t_3	t_4	Mean*
<i>I</i>	-0.40	—	-1.56	+0.25	+1.00	0.00	-0.50	—	—	-0.29
<i>II</i>	+0.13	—	-1.14	-0.55	+0.57	+0.50	-0.13	—	—	-0.01
<i>III</i>	-0.63	—	-1.00	—	+1.43	-0.50	+0.67	—	—	-0.01
Mean	-0.30	—	-1.23	—	+1.00	0.00	+0.01	—	—	-0.104

* Excluding T_2 , T_5 , t_3 and t_4 .

29 Apr.-1 May 1941

	T_1	T_2	T_4	T_5	T_6	C	t_2	t_3	t_4	Mean†
<i>I</i>	+0.83	+0.17	-1.33	-0.14	+0.83	+0.57	-1.29	0.00	+0.75	+0.27
<i>II</i>	+0.43	+0.29	+0.73	—	+0.29	+1.17	+0.43	0.00	+0.25	+0.41
<i>III</i>	+0.33	+1.43	—	+1.17	+0.57	-0.86	+0.29	+0.75	+1.27	+0.54
<i>IV</i>	+1.00	—	-0.57	+0.71	+0.86	—	—	-0.29	+0.33	—
Mean†	+0.53	+0.63	—	—	+0.56	+0.29	-0.19	+0.25	+0.76	+0.405

† Excluding T_4 and T_5 .‡ Excluding *IV*.(b) Frequencies of sets with and without inflorescence primordia, with emerged-leaf numbers above and below the (approximate) median value. (Sowings *I* to *III* and duplicates A+B taken together.) Calculated values in brackets.

13-15 Mar. 1941

T_4			
Leaves:	4-5	6-7	Total
+infl.	3 (1.3)	0 (1.7)	3
-infl.	8 (9.7)	15 (13.3)	23
Total	11	15	26

 $P=0.064$

T_6			
Leaves:	4-5	6-7	Total
+infl.	1 (3)	5 (3)	6
-infl.	11 (9)	7 (9)	18
Total	12	12	24

 $P=0.078$

29 Apr.-1 May 1941

C			
Leaves:	4-5	6-7	Total
+infl.	3 (5.5)	8 (5.5)	11
-infl.	9 (6.5)	4 (6.5)	13
Total	12	12	24

 $P=0.050$

T_1			
Leaves:	4-5	6-7	Total
+infl.	0 (2.1)	5 (2.9)	5
-infl.	10 (7.9)	9 (11.1)	19
Total	10	14	24

 $P=0.047$

For leaves plus scales, only T_6 showed any appreciable deviation from proportionality and that only for 13-15 Mar. In this one case, however, the effect was large and very significant ($P=0.013$), all the six sets with inflorescence initials falling in the 16-21 total leaf class and 11/18 of those without such initials falling in the 13-15 class (cf. Table 7 (b)). This was clearly due to the mutual reinforcement of the effects of leaf number and scale number for T_6 , which were non-significant when considered separately. Both effects if real could be explained as due to the relatively large short-period fluctuation in temperature which occurred in the incubator used for treatment T_6 . In view of the positive correlation of emerged leaf number and set weight, the sets with higher leaf numbers tended to be bigger, and would therefore

have suffered less from the peak temperatures both as regards flower initiation and scale formation during storage treatment. It should be noted that in T_6 , significantly fewer scales were formed during storage than in C , and also fewer inflorescences though not significantly so by 13-15 Mar.

Dissections of plants from greenhouse treatments, and general discussion

The data for the scale numbers in the plants pickled at the end of the greenhouse treatments reveal little of interest, except that for comparable treatments high second-season temperature caused a lower scale number in almost every case. The means were: *H*, 6.7; *h*, 7.8. This is evidence that the temperatures in *H* were high enough to have a harmful

effect on leaf initiation. Apart from this, high scale number was associated throughout with high incidence of bulbing as would be expected.

The numbers of emerged leaves* at the end of the experiment are of some interest. They have already been briefly discussed (Heath, 1943*b*, p. 314), but the following additional considerations merit attention. None of the average effects of the five single factors reach significance when compared with the significant interactions in which they are involved; the average effects of day length and of greenhouse temperature already discussed (Heath, 1943*b*, p. 314), and also that of storage temperature, are highly significant ($P < 0.001$) compared with the random variance. Of the 2-factor interactions, two are highly significant ($P < 0.001$), namely, the interaction between day length and storage temperature previously described (Heath, 1943*b*, Table 4, p. 315) and that between day length and greenhouse temperature. The appropriate means for the latter are shown in Table 8. The interaction consists of a large

TABLE 8. *Five-factor experiment, 1941. Mean final numbers of emerged leaves on plants grown in greenhouse*

(For symbols see Table 1, p. 174)

Means over all levels of germination date, temperature in first season, and storage treatment (i.e. from forty-eight tins of five plants each)

	<i>H</i>	<i>h</i>
<i>D</i>	8.6	11.3
<i>d</i>	16.4	17.1
S.E. of a single mean	= 0.24	
Significant diff. required for $P 0.05$	= 0.59	
Significant diff. required for $P 0.01$	= 0.78	

difference between the number of leaves emerged at high (*H*) and low (*h*) temperature in the long days (*D*), as compared with a very small difference in short days (*d*). This was doubtless due to the earlier and more rapid prevention of leaf emergence by bulbing in the long days at high temperature (*DH*) than at low (*Dh*). Even in short days, however, there is a significant difference between the leaf numbers at high (*dH*) and low (*dh*) temperature, and although this may have been an effect of the small amount of bulbing that did occur (Heath, 1943*b*, Text-fig. 3, p. 313) it again suggests a harmful effect of the high temperature as in the data for scale number. One of the 3-factor interactions, that between first season temperature, germination date and storage temperature, just reaches the 5% level of significance, but the data will not be presented or discussed.

In Table 9 are shown the percentage incidences of inflorescence initials in the leaf-marked *f* sets

* These numbers include swollen scales formed in the first season, in so far as these could be counted by their short abortive laminae.

TABLE 9. *Main experiments, 1940-1. Dissections of leaf-marked sets 29 Apr.-1 May 1941*

(For symbols see Table 1, p. 174)

Percentage of sets (produced at low temperature *f*) with inflorescence primordia. (Each value based on eight sets)

	<i>C</i>	<i>t</i> ₃	<i>T</i> ₃	<i>T</i> ₄
<i>I</i>	50.0	12.5	0.0	25.0
<i>III</i>	37.5	12.5	0.0	0.0
<i>IV</i>	37.5	0.0	0.0	12.5
Mean	41.7	8.3	0.0	12.5

pickled 29 Apr.-1 May 1941. These are undoubtedly higher than in the corresponding unmarked sets owing to the difference in weight, but provide an estimate of the probable treatment effects among the latter at the time they were planted in the greenhouse. No inflorescence initials were found in *F* sets.

As already mentioned (Heath, 1943*b*, p. 315), six inflorescences emerged in *dh* during the experiment (from *fC* and *ft*₃ plants) and one abortive inflorescence in *dH* from an *fC* plant. In addition to these many of the *f* plants were found on dissection to contain a suppressed inflorescence.† In every case this had been pushed to one side by the development of the axillary bud and the scape had generally elongated somewhat—sometimes to as much as 2 cm. The distribution of inflorescences (suppressed plus emerged) is shown in Table 10. The storage treatment means

TABLE 10. *Five-factor experiment, 1941. Percentage of sets planted in greenhouse which showed inflorescences (suppressed or emerged) on dissection at the end of the experiment. Also percentage with emerged inflorescences (in brackets). f sets only*

(For symbols see Table 1, p. 174)

Means over all levels of germination date (i.e. from six tins of five plants each)

	<i>C</i>	<i>t</i> ₃	<i>T</i> ₃	<i>T</i> ₄	Mean
<i>DH</i>	17.9	7.2	0.0	11.1	11.7
<i>dH</i>	18.2 (4.6)*	3.9	0.0	5.6	9.4
<i>Dh</i>	14.3	10.5	0.0	0.0	8.4
<i>dh</i>	31.8 (18.1)	10.4 (10.4)	0.0	0.0	14.5
Mean	20.6	8.0	0.0	4.2	—

* Abortive.

agree tolerably well with those in Table 9, though the percentages are lower, as would be expected from the smaller size of the sets and from the ease with which suppressed inflorescences may be overlooked. Analysis of variance shows a significant effect ($P < 0.05$) of storage treatment, but the effects of the greenhouse treatments are entirely non-significant. This lack of evidence for effect of day length or second season temperature on numbers of inflorescences, coupled with the approximate agreement

† A single *F* plant, from treatment *FICdh*, also showed a suppressed inflorescence.

of the data with the values in Table 9 strongly suggests that no further inflorescence initiation occurred after planting. It appears, then, that the effects of the greenhouse treatments on flowering were confined to inflorescence *emergence*; and that suppression occurred in all such treatments, even to some extent in the short day-low temperature treatment *dh*. The available data bearing on the mode of action of such suppression will now be considered.

It has been noted (Heath, 1943*b*, p. 316) that conditions of day length and temperature favourable to bulbing tend to prevent flowering. In *dh*, the six inflorescences emerged between 26 May and 10 June, before any bulbing had occurred in that treatment. In the long day-low temperature treatment, *Dh*, on the other hand, no inflorescences emerged, but twenty-four out of the sixty *fC* and *ft₃* plants had bulbed before 10 June. Clearly, therefore, in treatment *Dh* the long days inhibited emergence, either by means of a direct effect transmitted from the exposed leaves to the inflorescence initial or, acting indirectly, by means of the bulb development that they promoted. Heath & Holdsworth (1943) have shown that at temperatures low enough to prevent or greatly delay bulbing, long days actually accelerate inflorescence emergence. Presumably, therefore, in the present experiment, before 10 June, the inhibition in *Dh* was due to the bulbing. After 10 June a sudden rise of temperature occurred (Heath, 1943*b*, Text-fig. 1), and in the next fortnight bulbing became general among the *C* and *t₃* plants in *Dh* (Heath, 1943*b*, Text-fig. 3); on the above hypothesis this bulbing would sufficiently account for the *complete* suppression of emergence, but the possibility of a direct high-temperature effect should also be borne in mind. In *dh*, within a week of the rise of temperature, two of the six recently emerged inflorescences began to die off and a third died some weeks later; no further inflorescences emerged, but some plants showed definite and persistent bulbing, and every suppressed inflorescence subsequently found on dissection was borne by such a plant. Again, therefore, any possible effect of high temperature in suppressing inflorescence emergence was confounded with bulb development. The high temperature treatments *DH* and *dH* may now be considered: in the former, even more rapid and general bulb development occurred than in *Dh*; but in *dH*, out of five plants found to contain suppressed inflorescences, only two had shown definite bulbing during the season. The other three showed on dissection very slight swelling of one leaf base, but this was not sufficient to give the plants the external appearance of definite bulb development, and subsequent work has shown that *slight* bulb development does not prevent inflorescence emergence. It thus seems most probable that high temperature exerts a direct effect, independent of bulbing, in suppressing the emergence of inflorescence initials already present in the sets. Heath & Holdsworth (1943) found a similar

direct effect of high temperature in preventing inflorescence *initiation* in growing plants.

High temperature also prevents the initiation of inflorescences in stored sets, as is shown by the data for treatment *T₃* in Table 6. Here, not only was initiation completely prevented during the high-temperature treatment, but there was also a large after-effect, for no inflorescence primordia were found 6–7 weeks later and practically no flowering occurred in the field (Heath, 1943*a*, Table 7, p. 218). High temperature for the first 8 weeks of storage (*T₂*) also exerted a remarkable after-effect, for no inflorescence initials were found 14 weeks later in mid-March, and even by the end of April the percentage of sets with initials was very much lower than in the *C* treatment. Here, too, the final incidence of flowering in the field was considerably reduced. All the high-temperature storage treatments (*T₁*–*T₆*) at least *delayed* initiation, for all of them showed significantly fewer initials than the *C* treatment in the dissections of 29 Apr.–1 May, and this is borne out by the field results (Heath, 1943*a*, p. 218).

Another possible example of an after-effect of high temperature on inflorescence initiation is provided by the *F* sets, in which no initials were found on dissection. These also gave practically no flowering in the field (Heath, 1943*a*, p. 218) and only one (suppressed) inflorescence in the greenhouse. Unfortunately, this effect is confounded with a size effect (Heath, 1943*b*, p. 315), and further investigation is needed. The effect of germination date on initiation (p. 180) is also confounded with size differences.

Low-temperature storage prevented inflorescence initiation during treatment (Table 6 (*a*), *t₃* and *t₄*) and exerted after-effects of some complexity. Thus treatment for 22 weeks (*t₃*), or for the first 8 weeks only (*t₄*), resulted in reduced initiation (compared with *C*) by the end of April and reduced flowering in the field. On the other hand, low temperature during the last 8 weeks (*t₄*), which prevented initiation during treatment, resulted in a very large *increase* during the ensuing 6–7 weeks at normal temperatures. At the end of this period the incidence of initials had already practically caught up with that in the *C* treatment (Table 6 (*b*)) and appeared likely to surpass it in view of the much greater rate of increase (Table 6 (*c*)). That this actually occurred is suggested also by the fact that *t₄* gave more flowering in the field than *C*, though not significantly so (Heath, 1943*a*, p. 219). The implications of this effect, if real, have already been discussed (Heath, 1943*b*, p. 316).

The effects of high- and low-storage temperatures on initiation of new scales at the growing point appear to have been in the main direct; there is little evidence of after-effects except that there was a tendency for sets from treatments preventing initiation to produce new scales more rapidly on returning to normal temperatures. This was especially so in *fT₃*, *fT₆*, *fT₃* and *ft₄* (Table 5 (*b*)). In the case of *ft₄* this effect somewhat resembles that already noted for flower

initiation. As mentioned earlier (p. 180) the results of dissections in March indicate that most of the scale initiation occurring at normal temperatures between mid-October and mid-March takes place in the last 8 weeks of that period, since high- or low-temperature treatment in the early part of storage was without significant effect. The contrast between the scale initiation in ft_3 and Ft_3 , and that between FT_3 and fT_3 (p. 180), indicates, as already suggested, some degree of acclimatization of the f and F sets to low and high temperature respectively. In the T_3 treatment, the initiation of a significant number of scales in F sets but not in the f sets is the more remarkable since the former suffered a much greater loss of weight in store (Heath, 1943*a*, Table 4, p. 215).

Apart from the question of treatment effects, the data from dissections have provided information as to the normal internal structure of onion sets and the changes which occur therein. The almost constant mean number of three swollen scales in the different weight classes of Bedfordshire Champion sets (Table 4) has been found subsequently to apply very generally to sets and even to large onions. Boswell (1923), describing the mode of sprouting of large bulbs of Yellow Globe Danver onion, says: 'The number of these leafless scales varies but is usually 8-10: . . . Counting from the outside the first three scales show practically no growth at any time.' The three scales referred to are presumably the 'swollen scales' of this paper. Even the total scale number has been found to vary little over a wide range of set weight (Tables 2, 4). This indicates that the better yielding qualities of large sets (in the absence of bolting—Thompson & Smith, 1938) are due rather to size of scales (and possibly to the number and size of swollen leaf bases) than to the number of leaf initials ready for emergence.

As pointed out earlier (p. 178), large set size cannot be caused by high total scale number, since nearly all

the variation in number is in the small unswollen scales; it must be mainly due to the size of the swollen scales and to swollen leaf bases. This being so it is perhaps not surprising that no relation could be established between inflorescence initiation and number of scales (p. 181)—the mode of operation of the 'size effect' on flowering would not be expected to lie here. On the other hand, high leaf number being associated with high weight (p. 177) would be expected to be associated also with much initiation of inflorescences; a significant effect of this type was demonstrated for the 'common' storage treatment C and also for T_1 (Table 7 (*b*)). The 'size effect' may therefore be a matter of number of emerged leaves and the statement must be withdrawn that: 'It does not appear to be a question of the number of leaves emerged or initiated...' (Heath, 1943*b*, p. 315).

A tendency for inflorescence initiation to be associated with a high total number of leaf initials formed since sowing (leaves plus scales) was found for one storage treatment (T_6) only, and here a plausible explanation is possible (p. 182). It appears probable, however, that as in cereals there is a minimal leaf number for such initiation, probably 12-14. No evidence of a maximal number, such as also occurs in cereals, was obtained in this experiment; subsequent work has shown it to be not less than 34 if it exists (p. 181).

In general it may be said that the data from the dissections have confirmed and considerably extended conclusions reached from the external responses of the experimental plants.

It is a pleasure to thank Prof. F. G. Gregory, F.R.S., for his constant encouragement and interest. Acknowledgement of the assistance given by Miss E. Bruck is made in the text. While engaged in this investigation P. B. Mathur was holding an 1851 Exhibition Fellowship.

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EXPLANATION OF PLATE 7

Fig. 1. Hand-cut longitudinal median section of a 6-leaved onion set from treatment *IfA*, pickled 11 Oct. 1940 before storage treatment. Counting from the outside (left) the following parts may be seen: the lower portions of one shrivelled leaf base, one swollen leaf base, two swollen scales and two unswollen scales; then the sheathing base of another unswollen scale; finally the four youngest scales and the terminal growing point. Note the central 'pith', free from vascular bundles, in the stem below the growing point, and also the tip of each scale lying in the pore of the next older one. These characteristics show that the section is median and in the correct plane. $\times 10\frac{1}{2}$. (Preparation by O.V.S.H.)

Fig. 2. Central portion of the same, $\times 31\frac{1}{2}$, showing the growing point in the vegetative state.

Fig. 3. Hand-cut longitudinal median section of an onion set from treatment *IIfCA*, pickled 14 Mar. 1941, at the end of storage treatment. Central portion showing the terminal growing point developed into an inflorescence initial with scape and spathe. $\times 17$. (Preparation by Miss E. Bruck.)

Fig. 4. Microtome section of an onion set fixed 20 May 1942, showing the terminal growing point developed into an inflorescence initial with scape, spathe and a bract; also the lateral bud in the axil of the innermost scale surrounding the inflorescence. (Preparation by P.B.M.)

(Received 10 March 1944)

Soil conditions and the take-all disease of wheat

VIII. Further experiments on the survival of *Ophiobolus graminis* in infected wheat stubble

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Assimilable nitrogen in various forms prolonged the life of *Ophiobolus graminis* in infected wheat straw, whether added directly to the straw or to the surrounding soil. When the infected straws were buried in washed quartz sand, 0.5 g. nitrogen per 100 g. air-dry straw was the optimum dressing for longevity of *Ophiobolus*. Addition of sodium phosphate did not significantly increase longevity.

Nitrogen is considered to prolong the life of *Ophiobolus* by enabling the mycelium to form new branch hyphae, which can explore unexhausted parts of the substrate; it is suggested that aged mycelium dies from carbohydrate starvation, through exhaustion of the zones of enzymic erosion around the hyphae. This hypothesis is supported by the extended life of the fungus in infected straws that were shaken twice weekly in 3 % dextrose solution.

Ophiobolus was found to survive longer in infected straws buried in a fallow soil than in the same soil under oats, mustard or trefoil; this finding suggests the use of catch crops as competitors with *Ophiobolus* for soil nitrogen.

Previous experiments on the survival of *Ophiobolus graminis* in infected wheat straw buried in soil (Garrett, 1938, 1940a) indicated that the fungus disappeared most quickly under those soil conditions, such as good aeration, adequate moisture and medium to high temperature, favouring general microbiological activity. *Ophiobolus* disappeared particularly rapidly under conditions of acute nitrogen shortage in the soil surrounding the infected straw, as when nitrogen-poor organic materials were incorporated with the soil. Conversely, the fungus survived for exceptionally long periods when provided with an ample supply of available nitrogen. The paramount effect of nitrogen in determining longevity of *Ophiobolus* was interpreted on the assumption (Garrett, 1940a, 1942) that survival of the fungus in the infected plant tissue depends upon its ability to form fresh hyphae. The average nitrogen content of the artificially infected straw employed in these ex-

periments was only 0.4 %, so it is reasonable to suppose that the continued mycelial development of *Ophiobolus* in the cells of the straw would have been limited by the supply of available nitrogen from the soil.

No general review will be made of other investigations on survival of *Ophiobolus* in the soil, as these have recently been discussed elsewhere (Garrett, 1942).

EXPERIMENTAL

The technique employed in the following experiments has already been described (Garrett, 1938).

Experiment I. This experiment was designed to compare the effect of three forms of nitrogen, calcium nitrate, ammonium nitrate, and ammonium sulphate, at three different rates of application, 0.225, 0.45, and 0.90 g. nitrogen per 100 g. air-dry straw. The air-dry weight of the fifty infected straws buried in

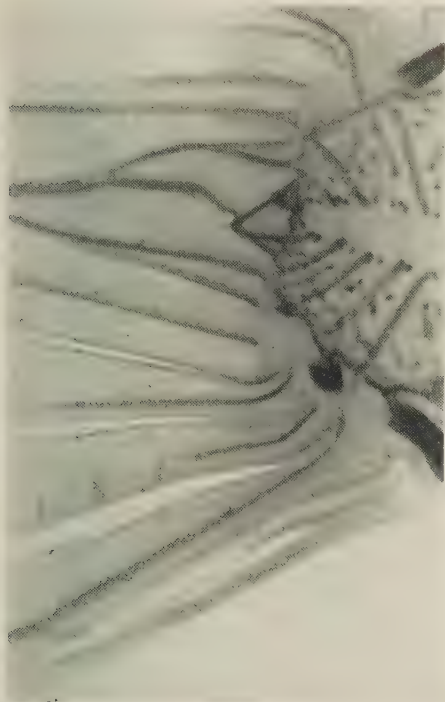


Fig. 1

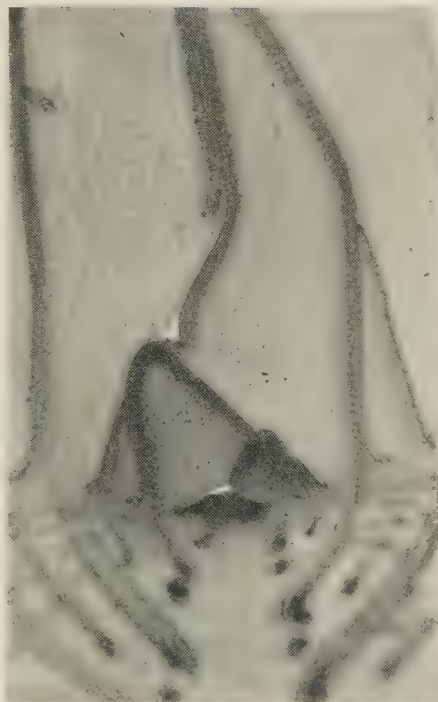


Fig. 2

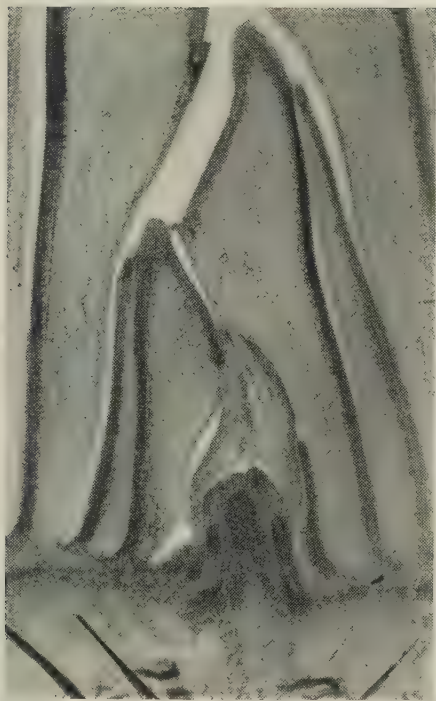


Fig. 3

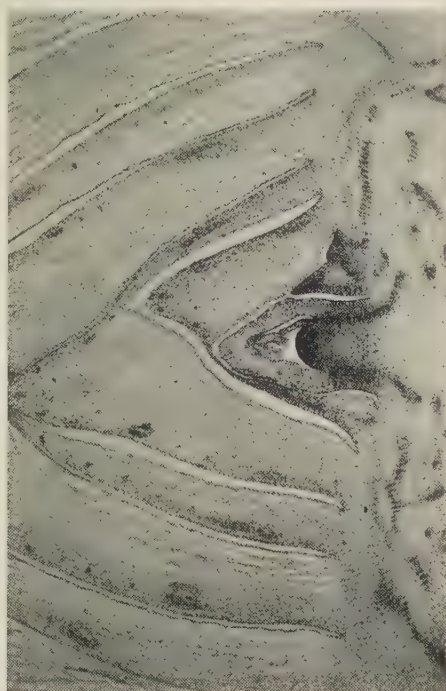


Fig. 4

each tumbler averaged 5.5 g., so that amounts of nitrogen added per tumbler were 0.0125, 0.025 and 0.05 g. respectively. The soil employed in this experiment was from one of the Rothamsted arable fields; 200 g. air-dry soil was allotted to each tumbler. Moisture content of the soil was maintained at 50% saturation by weekly addition of distilled water; evaporation was checked by provision of a sand mulch. The tumblers were kept on a bench in the laboratory, the mean weekly temperature of which varied from 16 to 20° C. At 3-weekly intervals from 10 weeks onwards, a sample of two tumblers, comprising 100 straws, was taken for determination of viability of *Ophiobolus* by the wheat seedling test (see Table 1).

Comparison of survival of *Ophiobolus* in the untreated soil with that in the others demonstrates the pronounced effect of nitrogen in promoting longevity of the fungus. The effect was consistently greatest with calcium nitrate, and least with ammonium sulphate. Of the three rates of application tested in

before being autoclaved for 1 hr. at 2 atm. pressure. The flasks were inoculated with small agar inoculum disks of *Ophiobolus* and incubated for 14 days at 25° C.; sand and straws were then thoroughly shaken up together, to distribute the fungus mycelium, and the flasks returned to the incubator for a further 16 days. In order to conserve nutrients, the straws were not washed when taken out of the culture flasks, but were freed of sand by dry sieving. This modified technique has not eliminated variation in nutrient content of the original straw, but has eliminated additional variability formerly introduced by soaking of the straws in string bean decoction, the nutrient composition of which was both variable and unknown.

For this particular experiment, but not for any of the subsequent ones reported in this paper, certain nutrients were dissolved in the distilled water added to the dry straw and sand at the time of flasking, in order to study the effect of variation in nutrient content of the straw on survival of *Ophiobolus*. Half the straws in the experiment received 5 g. dextrose

TABLE 1. *Percentage of straws containing viable Ophiobolus mycelium*

	Weeks						
	10	13	16	19	22	25	28
No nitrogen	95	79	74	65	47	35	24
0.225 g. N/100 g. straw as Ca(NO ₃) ₂	91	85	84	80	78	67	49
0.45 g. N/100 g. straw as Ca(NO ₃) ₂	95	84	97	93	90	88	79
0.90 g. N/100 g. straw as Ca(NO ₃) ₂	97	90	96	85	78	82	68
0.225 g. N/100 g. straw as NH ₄ NO ₃	92	87	80	82	72	52	41
0.45 g. N/100 g. straw as NH ₄ NO ₃	93	84	86	88	82	86	71
0.90 g. N/100 g. straw as NH ₄ NO ₃	94	86	80	79	79	73	71
0.225 g. N/100 g. straw as (NH ₄) ₂ SO ₄	90	80	82	63	60	—	—
0.45 g. N/100 g. straw as (NH ₄) ₂ SO ₄	92	85	76	86	73	61	64
0.90 g. N/100 g. straw as (NH ₄) ₂ SO ₄	91	85	77	77	58	44	32

this experiment, 0.45 g. per 100 g. air-dry straw was the optimum for promoting survival. The maximum rate, 0.9 g., was evidently supra-optimal, and appears to have had a slightly toxic effect, which was most pronounced with ammonium sulphate as the source of nitrogen.

Experiment II. This experiment was designed to test the effect of variation in nutrients within the original inoculated straw on subsequent survival of *Ophiobolus*. For this and subsequent experiments reported in this paper, the original technique* for the preparation of infected straws was simplified as follows. The air-dry cut straw, in 32 g. lots, was placed in 500 c.c. conical flasks and covered with 350 g. dry sand; 120 c.c. distilled water was then added, and the flasks allowed to stand overnight

per 100 g. air-dry straw; in each batch, with and without dextrose, half the number received 0.5 g. nitrogen as sodium nitrate per 100 g. straw. After inoculation with *Ophiobolus* and incubation, the infected straws were buried in lots of fifty in tumblers of washed quartz sand, maintained at a moisture content of 50% saturation; the mean weekly temperature of the laboratory varied from 14 to 21° C. A sample of three tumblers, comprising 150 straws, was taken every 4 weeks for determination of *Ophiobolus* viability; results are given in Table 2.

TABLE 2. *Percentage of straws containing viable Ophiobolus mycelium*

	Weeks					
	4	8	12	16	20	24
No treatment of straw	97	81	38	50	4	13
5 g. dextrose/100 g. straw	98	92	49	33	—	—
0.5 g. N/100 g. straw	100	95	86	100	43	60
5 g. dextrose + 0.5 g. N/100 g. straw	100	98	75	77	72	53

* In the original technique, the batch of cut straws for an experiment was first boiled for 3 hr. in a string-bean decoction at the rate of 1 lb. beans in 4 l. tap water to every 4000 straws. After autoclaving in 2 l. conical flasks, the straws were heavily inoculated with a culture of *Ophiobolus* on sand + 3% corn-meal medium.

The addition of nitrogen to the straw has produced just as marked an increase in the longevity of *Ophiobolus* as when it was added to the soil in earlier experiments. The particularly rapid disappearance of the fungus from the untreated straws in this experiment, as compared with that in Exp. I and other previous experiments, may be attributed to the omission of bean decoction in preparation of the infected straws, and to the use of washed quartz sand instead of soil as the surrounding medium in the tumblers. The addition of 5% dextrose to the straws does not seem significantly to have affected longevity of *Ophiobolus*.

Experiment III. Consideration of the average phosphate content of wheat straw (0.18% P_2O_5), according to the compilation by Beeson, 1941) suggested that, in the presence of ample nitrogen, the amount of available phosphate might limit survival of *Ophiobolus*. In a small preliminary experiment, infected straws prepared by the new technique were buried in tumblers of washed quartz sand, to which were added nitrogen as sodium nitrate at concentrations of 0, 0.25, 0.50 and 0.75 g. nitrogen per 100 g. air-dry straw, and phosphate as sodium phosphate at concentrations of 0, 0.0625, 0.125, and 0.25 g. P_2O_5 per 100 g. straw, in all possible factorial combinations. The results showed that 0.5 g. nitrogen was the optimum concentration for survival of *Ophiobolus*, but that the 0.0625 and 0.125 concentrations of P_2O_5 exerted no consistent effect. At the maximum rates, both nitrogen (0.75 g.) and phosphate (0.25 g. P_2O_5) reduced survival of *Ophiobolus*, suggesting a toxic effect; such toxicity would be likely to occur at a lower level of the nutrient in quartz sand, a non-colloidal medium, than in soil. With the information derived from this preliminary experiment, another was set up in which the effect of sodium phosphate at concentrations corresponding to 0.0625 and 0.125 g. P_2O_5 per 100 g. air-dry straw was tested in the presence of 0.5 g. nitrogen as sodium nitrate. Washed quartz sand, at a moisture content of 50% saturation, was again used as the tumbler medium; the mean weekly temperature of the laboratory varied from 14 to 21° C. At the first and last sampling, four tumblers (200 straws) were taken from each series, and at the two intermediate samplings five tumblers (250 straws) were taken (see Table 3).

Statistical analysis of the results summarized in Table 3 has shown that phosphate failed to exert any significant effect on longevity of *Ophiobolus*. There was a rather wide variation in percentage survival of the fungus in the four or five tumblers taken from each series at every sampling. This variation may have been associated with the use of quartz sand instead of soil as the tumbler medium, as such wide variation has seldom been encountered when soil has been used. The consistency of results obtainable in soil is shown, for instance, in Table 5. It is possible

that the lack of any buffering mechanism in quartz sand may have contributed to the wide variation between results from individual tumblers in this experiment. Even so, the failure of phosphate to produce any significant increase in longevity of *Ophiobolus* was unexpected, in view of Chang's (1939) report that decomposition of composted wheat straw by a general soil microflora proceeded substantially further in the presence of extra phosphate, in addition to the usual supplement of nitrogen. It is true that Chang employed potassium phosphate in his experiments, and not sodium phosphate as here, but this difference is unlikely to be of relevant significance, in view of the high average potash content of wheat straw (approx. 1%, according to Beeson, 1941).

TABLE 3. Percentage of straws containing viable *Ophiobolus* mycelium

	Weeks			
	16	24	28	32
No nutrients added	13	12	14	4
0.5 g. N/100 g. straw	69	32	70	27
0.5 g. N + 0.0625 g. P_2O_5 /100 g. straw	69	45	52	21
0.5 g. N + 0.125 g. P_2O_5 /100 g. straw	82	59	70	55

Experiment IV. This experiment was designed further to elucidate the effect of nitrogen in promoting survival of *Ophiobolus*. It seems reasonable to assume that this is merely a particular instance of the general observation that decomposition of wheat straw proceeds only very slowly in the absence of supplementary nitrogen. Additional nitrogen is necessary for the synthesis of microbial protein by the micro-organisms engaged in decomposing the plant tissue (Waksman, 1931). The mechanism of this decomposition, originally the subject of pioneer work by Henneberg (1922), has been demonstrated by Baker and his collaborators (for a general review, see Baker 1939a) by means of direct microscopical observation. Baker has taken advantage of the fact that the cellulose and hemicellulose components of the cell wall are birefringent; under microscopical examination in polarized light, disintegration of the cell wall is revealed by loss of birefringence. Baker has correlated this with changes in reaction to micro-chemical reagents and dyes. Using these techniques, he has elegantly demonstrated the occurrence of zones of enzymic erosion in the cell wall around individual bacterial cells, and also around fungal hyphae (Baker, 1939b). These observations were initiated in a series of investigations on decomposition of plant tissues in the alimentary canal of different herbivorous animals, but have been extended by Baker to a study of cell-wall disintegration in animal faeces and in composts. Similar enzymatic cavities around fungal hyphae were demonstrated by Bailey & Vestal (1937) in a study of timber rots.

These observations by Baker suggest that the mycelium of *Ophiobolus* may die from carbohydrate starvation when it has exhausted the zones of enzymic erosion around the hyphae. In the presence of available nitrogen, however, *Ophiobolus* can form new branch hyphae, which can explore unexhausted parts of the substrate, and thus the life of the fungus is preserved for a further period. In the experiment now to be described, this hypothesis was tested by providing *Ophiobolus* in the infected straw with a regularly maintained supply of dextrose, but no additional nitrogen. To afford this, the infected straws (originally 730 in number) were shaken up twice weekly in 1.5 l. of 3% dextrose solution. The shaking was carried out for 20 min. in an end-over-end mechanical shaker. In order to ensure adequate inoculation of the straw with the soil microflora, 6 c.c. of fresh garden soil was added to each shaking bottle at every shaking for the first 3 weeks of the experiment. A control series was shaken in an equal volume of distilled water. A third series was shaken in a solution containing 0.03% sodium nitrate and 0.003% potassium phosphate. In between shakings, the straws were kept covered in 7 in. diameter unglazed flower pots; the mean weekly temperature of the laboratory varied from 13 to 18°C. Samples of 100 straws were taken from each series at 3-weekly intervals (but the final sample was taken at 13 weeks) (see Table 4).

TABLE 4. *Percentage of straws containing viable Ophiobolus mycelium*

	Weeks			
	3	6	9	13
Distilled water	100	45	17	3
3% dextrose	99	74	39	1
Nitrate + phosphate	100	98	34	2

The results of this experiment have agreed with expectation from the hypothesis advanced above, in so far as dextrose has clearly retarded decline of *Ophiobolus*, even if only for a limited period. It is possible that the fungus may have been inhibited from infecting the test wheat seedlings in some of the straws by an accumulation of fungistatic or even fungicidal by-products of dextrose respiration. Although the straw container was an unglazed flower pot, evidence of incomplete respiration of the dextrose was offered by development of a strong odour of butyric acid, which had become particularly marked at the 6-week sampling. It is not surprising that *Ophiobolus* did not survive for longer in the straws receiving nitrate + phosphate, as determination of nitrogen content of the straw at the end of the experiment (see below) showed that nitrate content of the shaking solution had been too low.

In criticism of this experiment, it can be argued that the effect of the dextrose on survival of *Ophiobolus* might have been wholly indirect, through the

encouragement of free-living nitrogen-fixing organisms, by provision of optimal conditions for their activity (Waksman, 1931). Even assuming that such organisms had fixed substantial amounts of nitrogen in the course of this experiment, it is doubtful whether *Ophiobolus* would have benefited much thereby, as in the presence of excess dextrose, the nitrogen fixers would probably themselves assimilate all the nitrogen that they fixed. Moreover, other micro-organisms in the straws would compete with *Ophiobolus* for any nitrogen that might be set free by the nitrogen-fixers, and recently liberated nitrogen might also be lost during the twice-weekly shaking in dextrose solution. Such considerations apart, chemical analysis of the straws has failed to show any significant accretion of nitrogen in the dextrose series. At the conclusion of the experiment, after 13 weeks, the straws were oven-dried and their total nitrogen content determined. Some 340 straws remained over in each series for this analysis; the nitrogen content per 300 straws was 0.080 g. in the water series, 0.085 g. in the dextrose series, and 0.115 g. in the nitrate series.

Although the results of this experiment agree with expectation from the simple hypothesis advanced above, it is necessary to point out that the effect of nitrogen in promoting longevity of *Ophiobolus* can be explained in other ways. For instance, nitrogen might permit development in the straws of certain other micro-organisms, from the activities of which *Ophiobolus* directly benefited. Against this hypothesis of synergism, and in favour of the hypothesis postulating a direct effect of nitrogen on *Ophiobolus*, may be cited the observation that nitrogen accelerated decomposition of wheat straw by this fungus in pure culture, presumably by permitting a denser development of mycelium (Garrett, 1940b).

Experiment V. The ultimate objective of these and previous experiments has been the development of practical measures for accelerating the disappearance of *Ophiobolus* from the soil. An attempt has therefore been made to exploit the discovery that longevity of the fungus depends upon the supply of nitrogen from soil to straw. The striking acceleration in decline of *Ophiobolus* produced by incorporation of rye-grass meal and other nitrogen-poor organic materials with the soil at first sight seemed to be of potential practical value. But the incorporation even of 1% finely divided organic material with the soil, though easy in tumblers, is difficult to carry out in the field. Moreover, the take-all disease can be controlled by a relatively short rotation, especially if advantage be taken of certain other precautions that can be fitted without inconvenience into good farming practice (Garrett, 1942). Only those precautions that are likely to pay their way through increased crop yields even in the virtual absence of *Ophiobolus* from the soil are likely to be adopted by the farmer.

Consideration of the fact that nitrates accumulate in fallow soils suggested that *Ophiobolus* might die

out more quickly in soil under a non-susceptible crop than in soil left fallow. If this were so, then it might pay the farmer to cover the soil even for relatively short periods of fallow with a catch crop or green manure crop. Of particular interest in this connexion is a system followed by certain English farmers for continuous or semi-continuous barley growing. Seed of trefoil (*Medicago lupulina*) is drilled along with the barley; after barley harvest, the well-established plants of trefoil make a luxuriant autumn growth, and are ploughed under in winter as a preparation for the next barley crop. Although the take-all disease is frequently seen in second or third consecutive barley crops, it has not so far been encountered in barley grown under this system.

An experiment was therefore designed to test this hypothesis of competition between *Ophiobolus* and the roots of crop plants for nitrogen. Although a brief summary of the results has already been published (Garrett, 1943), particulars of technique and the detailed results will be relevant here. The experiment was carried out in standard seed boxes, $14 \times 8\frac{1}{2} \times 3$ in., filled with a mixture of 3 vol. of

ence between these crop plants, but to the fact that the oats made a quicker start from the seed than did the other two crops. This experiment has thus demonstrated the possibility that crop plants may be used to compete with *Ophiobolus* for available soil nitrogen. There are many differences, however, between the soil environment in the boxes of this experiment, and that in the open field; confirmation of these results is now required by actual field experiment under normal farming conditions. Nevertheless, an attempt has been made to narrow the gap between these tumbler and box experiments and the projected field experiments, by means of two small nitrogen experiments with naturally infected wheat stubble, to confirm those made with artificially infected straw.

Experiments VI and VII. The infected stubble for Exp. VI was collected in late August 1941, and air-dried. Four months later, 160 well-infected tiller-bases were picked out, not too closely trimmed of roots, soaked in water, and buried in lots of forty in tumblers of quartz sand; two tumblers received sodium nitrate at the rate of 0.5 g. nitrogen per 100 g.

TABLE 5. *Percentage of straws containing viable Ophiobolus mycelium*

	Box 1	Box 2	Box 3	Box 4	Box 5	Mean
Fallow soil	50	68	67	83	71	68
Soil planted with trefoil	24	18	13	27	12	18
Soil planted with mustard	21	23	12	15	16	17
Soil planted with oats	6	3	0	7	2	4

Harpenden allotment soil and 1 vol. of potting sand. Total nitrogen content of the soil mixture was 0.21 %, and nitrate nitrogen 1.7 mg./100 g. soil. The boxes were divided into four lots, one of which was left fallow and the other three planted with trefoil, mustard and oats, respectively. Three rows of seeds were planted in each box; rows were 3 in. apart and seeds 2 in. apart. Several seeds were planted in each hole, but seedlings were thinned to one per hole as soon as they were well up. Seeds were planted on 18 and 19 May, and the boxes were kept outside; 3 weeks later, when the seedlings were up, fifty infected straws were inserted vertically into the soil of each box, in four rows (two rows together between the rows of seedlings). At burial of the straws, the oat seedlings were furthest advanced; the trefoil seedlings were still very small. Eight weeks after burial of the straws, 250 straws from five boxes in each series were tested for *Ophiobolus* viability; results are given in Table 5.

All differences between means are statistically significant except that between the trefoil and mustard series. The more rapid disappearance of *Ophiobolus* under oats than under mustard or trefoil is probably to be attributed not to any intrinsic differ-

air-dry straw. The moisture content of the sand was maintained at 50 % saturation; the tumblers were kept for 3 months in the laboratory, the mean weekly temperature of which varied from 16 to 21°C., and then the eighty straws of each series were tested for *Ophiobolus* viability. The infected stubble for Exp. VII was collected in late August 1943, and 200 well-infected tiller-bases were picked out as soon as possible, trimmed, soaked in water and buried in four tumblers of quartz sand; other experimental arrangements were similar to those of Exp. VI, except that the tumblers were kept in a 25°C. incubator, and the test for viability of *Ophiobolus* was made after 8 weeks. Results of both experiments are set out in Table 6.

TABLE 6. *Percentage of straws containing viable Ophiobolus mycelium*

	No addition	0.5 g. N/100 g. straw
Exp. VI, after 3 months	8	22
Exp. VII, after 8 weeks	15	38

These results confirm those obtained with the artificially infected straws.

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Factors influencing infection of the tomato by *Verticillium albo-atrum*. II

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Tomato plants receiving adequate supplies of mineral nutrient acquired great resistance to infection by *Verticillium albo-atrum* if the leaf-shoot ratio was reduced; this effect is attributed to reduction in carbohydrate content of the host. Wide variation in potash manuring did not affect susceptibility of tomatoes to *Verticillium*.

Previous work showed that the effect of *Verticillium albo-atrum* on tomato plants was greatly influenced by the amount of nitrogen supplied to them (Roberts, 1943). At low levels of nitrogen few plants became infected; they often showed no external symptoms and the fungus was usually confined to the roots. Increasing the supply of nitrogen increased both the number of plants that became infected and the severity of the disease. In this paper it is shown that other factors may be as important as nitrogen supply in determining susceptibility. Plants have been grown under conditions designed to reduce their rate of carbon assimilation; under such conditions they have been found to resist infection, even though supplied with ample nitrogen for maximum susceptibility. The reduction in susceptibility thus induced by curtailing photosynthetic activity may partially explain the improved control of wilt claimed by Bewley (1923) to follow shading of the glasshouse with whitewash, at the same time as the temperature is raised above 25°C.

METHODS

Tomato seedlings (var. Kondine Red) were transplanted into 8 in. pots containing glasshouse compost or allotment loam. In order that the roots might grow freely through the holes at the bottom of the pots, moss, instead of crocks, was used for drainage. Plants were inoculated when roots were visible in the drainage holes; they were then 10–12 in. high with the first flower buds developing. Earthenware pot-saucers, diameter 7 in. and depth 2 in., were half filled with soil over which was distributed 25 g. of a culture of *Verticillium* grown on sand plus 3% cornmeal. After filling the saucers to the top with more soil the potted plants were firmly settled in them. This method of inoculation had some advantage over the wooden flat method previously used (1943) in that a greater volume of soil was available for each plant. Nutrients were applied in solution to the pots. As external symptoms are not considered to be reliable indications of infection, sections of roots and stems of all plants were examined

microscopically for the presence of mycelium in the xylem elements.

EXPERIMENTAL

In a factorial experiment carried out previously (Roberts, 1943) on 324 plants with all possible combinations of nitrogen, phosphorus and potassium at three different levels, high, medium and low, the results showed a slight increase in the number of infected plants in the series which received no potash over those which received K_2SO_4 at the maximum

to be more frequent and severe in plants that have some advantage in lighting over their neighbours. In previous experiments (Roberts, 1943) when six plants growing in small pots were placed in wooden flats of inoculated soil, plants in outside positions were more frequently infected, developed symptoms earlier, and were more severely affected by the disease than the others. Remedies proposed by Bewley (1923) after an outbreak of wilt in glasshouses include raising the temperature to over $25^\circ C$. and shading the plants. Sabourova (1937) recommends planting cotton in closer stands affording denser shade as a method of control.

It was thought that the effect of shade in inducing recovery from *Verticillium* infection might be due to reduced carbon assimilation by the plant, rather than to reduced transpiration. In recent work on susceptibility of cotton seedlings to *Phymototrichum omnivorum* Blank (1940) found a higher incidence of root rot in seedlings just over a month old than in others of 3-4 weeks. The sharp increase in susceptibility in the older groups coincided with accumulation of carbohydrate in the seedling tissues.

Mean max. temperature during experiment May-July $27.23^\circ C$.

TABLE 1

Treatment per plant	Total no. of plants	No. of plants infected		
		Root only	Root and stem	Total
3.232 g. K_2SO_4	20	1	15	16
2.424 g. "	20	1	14	15
1.616 g. "	20	3	13	16
0.808 g. "	20	4	12	16
0 "	20	0	15	15

TABLE 2

Time of test	Days between first pruning and inoculation	No. of plants in each group	No. of plants infected in each of three treatments		
			Shoots removed	Untouched	Leaves removed
Sept.-Nov. 1942	1	15	5	5	1
Oct.-Mar. 1942-3	35	30	19	25	3
Apr.-June 1943	10	33	18	20	11*
Aug.-Nov. 1943	15	33	21	17	2
Total no. of plants infected in each group (out of 111)			63	67	17

* Five plants infected in roots only.

and half-maximum rates. To test this effect an experiment was planned with the maximum quantities of nitrogen (as $NaNO_3$) and phosphate (as NaH_2PO_4) that were employed in the previous experiment, but with potash (as K_2SO_4) ranging from zero to four times the maximum dressing used in the earlier experiments. Thus the actual quantities of nutrients supplied per plant were: 1.325 g. $NaNO_3$, 0.185 g. NaH_2PO_4 , and 0.3.232 g. K_2SO_4 . Twenty plants were allotted to each of the five potash treatments. Nutrient solutions were applied in two dressings, the first, one day after inoculation and the second, two days later. The results are shown in Table 1.

The high infection total of 77% shown in Table 1 for the whole experiment was expected from previous results with plants receiving the maximum nitrogen dressing. The results indicate that, at least when the supply of nitrogen and phosphate is sufficient for good growth of tomato plants, potash has no effect on susceptibility to *Verticillium*.

Carbohydrate content of tomato plants in relation to infection by *Verticillium*

It has been noticed in the course of experiments with *Verticillium* that there is a tendency for infection

Experiments to test the effect of altering the leaf-shoot ratio of tomato plants in an attempt to vary their carbohydrate content were carried out as follows: Plants growing in 8 in. pots were divided into three groups. In the first ('shoots removed') all secondary shoots and flowering spurs were removed in regular prunings, and finally, after inoculation, the terminal bud; this treatment resulted in development of large leaves with thickening and curling of the lower leaves. The second group ('untouched') was not pruned in any way. In the third group ('leaves removed') all but the youngest assimilatory leaves were stripped, but secondary shoots and flowering spurs were allowed to develop unchecked. These plants were pale green, with weak, thin stems: they did, however, produce a few normal, rather large fruits.

Plants were inoculated (in different tests) 1, 10, 15 and 35 days after pruning was first started, and the tests were made at all seasons of the year (Table 2).

From the difference in infection totals shown in Table 2 between the group 'leaves removed' and the other two groups, it appears that reduction in rate of carbon assimilation below a certain level has a pronounced effect in inducing resistance to *Verti-*

cillium. Under conditions most favourable for infection, such as those during the Apr.-June test, the difference in number of infected plants appears to be less pronounced, but five of the eleven infections shown in the 'leaves removed' group were confined to the roots of the plants. All these plants were given sufficient nitrogen to ensure susceptibility, as shown by the high percentage of infection in the 'shoots removed' and 'untouched' groups. In contrast with the pronounced effect of the 'leaves removed' treatment, the 'shoots removed' treatment has not altered the incidence of infection. It is possible that this treatment failed to increase carbohydrate concentration of the tracheal sap, or did not alter it sufficiently to affect the response of the fungus.

On the effect of the dual heat/shade treatment for checking the progress of wilt in a glasshouse *after* plants have become infected, Bewley (1923) found that the number of days wilted plants were kept in a shaded cucumber house at 25°C. before being returned to an average temperature of 20°C. determined the length of time before they again wilted. He concluded that 'temperature is a most important factor, while shading is valuable because it assists the plants by reducing transpiration'.

The treatment of the plants in the 'leaves removed' group in these experiments is certainly more drastic than any effect of shade on normal plants achieved by such means as whitewashing a glasshouse. The 'leaves removed' treatment not only reduced the number of plants that became infected, but it also

reduced the severity of the attack in infected plants. This suggests that even after entering a plant the fungus is unable to maintain itself unless it is supplied with sufficient carbohydrate; this might explain the effect of shading in controlling the disease in plants already infected. In this connexion it is interesting to note that Sabourova (1937) has recommended planting cotton in closer stands for control of wilt due to *V. dahliae*, on the assumption that reduced transpiration assists the plant. It is also possible, however, that shading might operate by reducing rate of carbon assimilation. In Uganda, Hansford (1940) reported that incidence of cotton wilt (due chiefly to *V. dahliae*) was diminished by closer spacing of rows, or by interplanting wide spacings with groundnuts or beans. He attributed this control to reduction in soil temperature, which was stated to vary at Bukulasa Station between 24 and 30°C., i.e. optimum for infection of this species of *Verticillium* (Ludbrook, 1933). In the Eastern Province of Uganda, on the other hand, where mean soil temperatures were higher than at Bukulasa, the wilt disease, though much less prevalent on the whole, tended to be more prevalent under shade.

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Factors influencing the development of cotton diseases

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(With 6 Text-figures)

The observations and experiments reported in the present paper were carried out in Suinin, Szechuan Province, West China, through the period 1938-41. Under local conditions, the incidence of most cotton diseases was observed to be influenced chiefly by air humidity. No matter whether it attacks the seedlings, mature plants, or bolls, such a disease requires a humid condition preceding the occurrence of an epidemic. For sore shin disease, soil moisture and soil temperature, however, have a more profound influence. Cyrtosis, which is a disorder caused by leafhopper injury, responds to the climatic factors in a different way. During the period of rapid reproduction of the leafhopper, higher temperatures result in the earlier appearance of the disease, while heavy rainfalls reduce the intensity.

Modification of the environmental conditions by varying the time of sowing influences the incidence of diseases occurring in both the seedling and mature stages of the cotton plant. Among seedling diseases, anthracnose is further proved to be influenced chiefly by air humidity in its prevalence. Sore shin disease, on the other hand, requires a moist soil condition for its spread and development. In the mature plant, late sowing increases the intensity of cyrtosis but reduces that of bacterial blight and anthracnose.

Modification of several cultural practices was also tested. Nitrogenous fertilizers generally increase the incidence of most diseases. Denser stand and closer spacing bring about a heavier infection in stem and leaf diseases but not in boll diseases. A mixed cropping system appears to have no effect on the fungal and bacterial diseases but reduces the intensity of cyrtosis when the crop interplanted with cotton is not susceptible to the infestation of leafhopper.

INTRODUCTION

Cotton plants in the northern part of Szechuan Province, West China, are subject to a number of diseases. The more common ones are bacterial blight (*Bacterium malvacearum* E.F.S.), anthracnose (*Glomerella gossypii* (South.) Edg.), sore shin (*Rhizoctonia solani* Kühn), alternariase (*Alternaria macrospora* Zimm. and *A. tenuis* Auct.), areolate mildew (*Mycosphaerella areola* Ehrlich & Wolf), boll rots (*Fusarium moniliforme* Sheld., *Diplodia gossypina* Edg., and a number of others), and cyrtosis, a disorder caused by the leafhopper (*Chlorita biguttula* Shir.). Since 1938, observations have been made on the seasonal development of these diseases, and their relation to the climatic factor have been recorded. Experiments were also made to ascertain the effect of the environmental conditions at the time of sowing and of other cultural practices on the prevalence of various diseases. The results, as presented in this paper, may help in the understanding of the developmental cycle of these diseases and in the intelligent application of control measures.

CLIMATIC FACTORS AND THE SEASONAL DEVELOPMENT OF COMMON DISEASES

The disease problem in the cotton district of this province is not essentially different from that in other parts of the world. For certain common

diseases, bacterial blight in particular, there is precise information regarding the factors influencing their development, while for others, though as common as anthracnose, such knowledge is lacking. Since 1938, the seasonal occurrence of several common diseases has been followed by extensive surveys made at intervals of 10 days throughout the growing season in Suinin, where the Provincial Cotton Experiment Station is located. In that area, the Asiatic cotton, *Gossypium arboreum* L., is extensively grown with occasional fields of an introduced upland variety, Delfos of *G. hirsutum* L. In general, sowing is done in May, anthesis begins in July, and picking in August. The results of the survey during the period 1938-41 are presented together with the meteorological records in Figs. 1-4. The essential climatic factors, namely, air temperature, humidity, and rainfall, are further presented in Fig. 5 in averages or totals of periods of 10 days to facilitate a comparison between different years.

A study of the meteorological data reveals that air humidity is a factor more variable between years than the other two and also the most influential to the development of nearly all the diseases concerned except cyrtosis and sore shin. Generally speaking, humidity, when temperature is not extreme, determines to a large extent the reproduction and longevity of the inoculum, the initiation and intensity of infection, and the survival of the organism. Tempera-

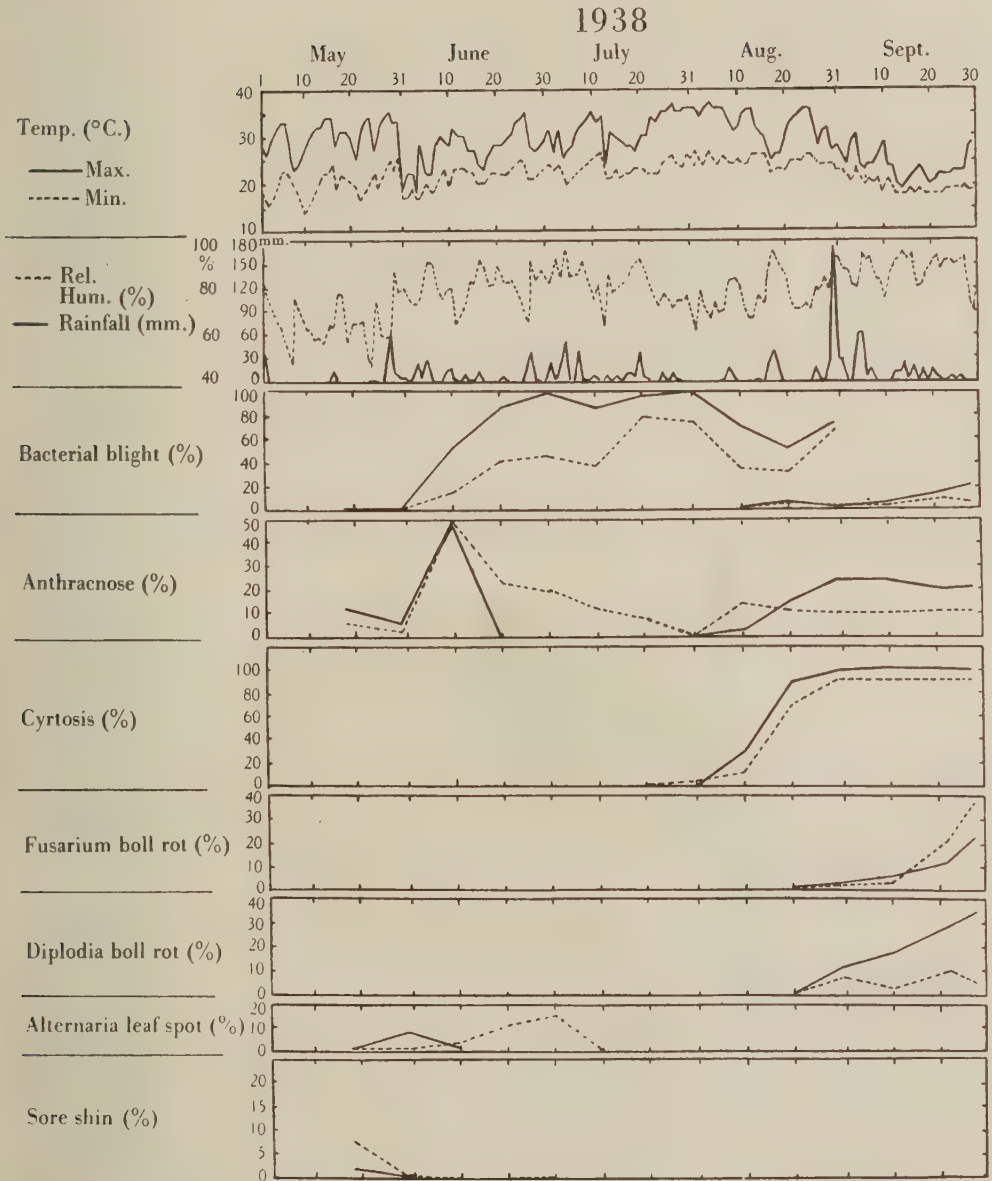


Fig. 1. Graphic summary of daily meteorological records and the development of common diseases of cotton. Suinin, Szechuan Province, China, 1938. — Delfos 531. - - - Local var.

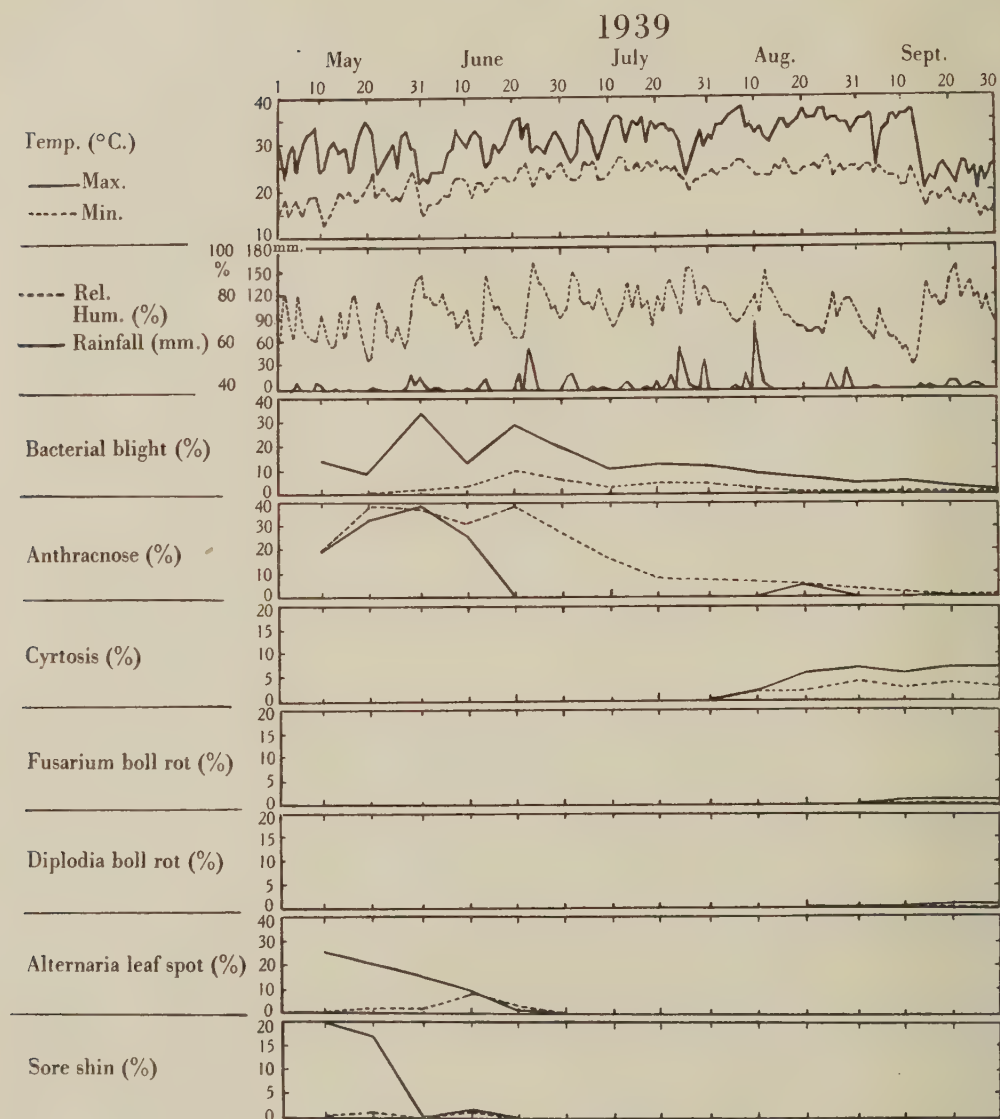


Fig. 2. Graphic summary of daily meteorological records and the development of common diseases of cotton. Suinin, Szechuan Province, China, 1939. — Delfos 531. - - - Local var.

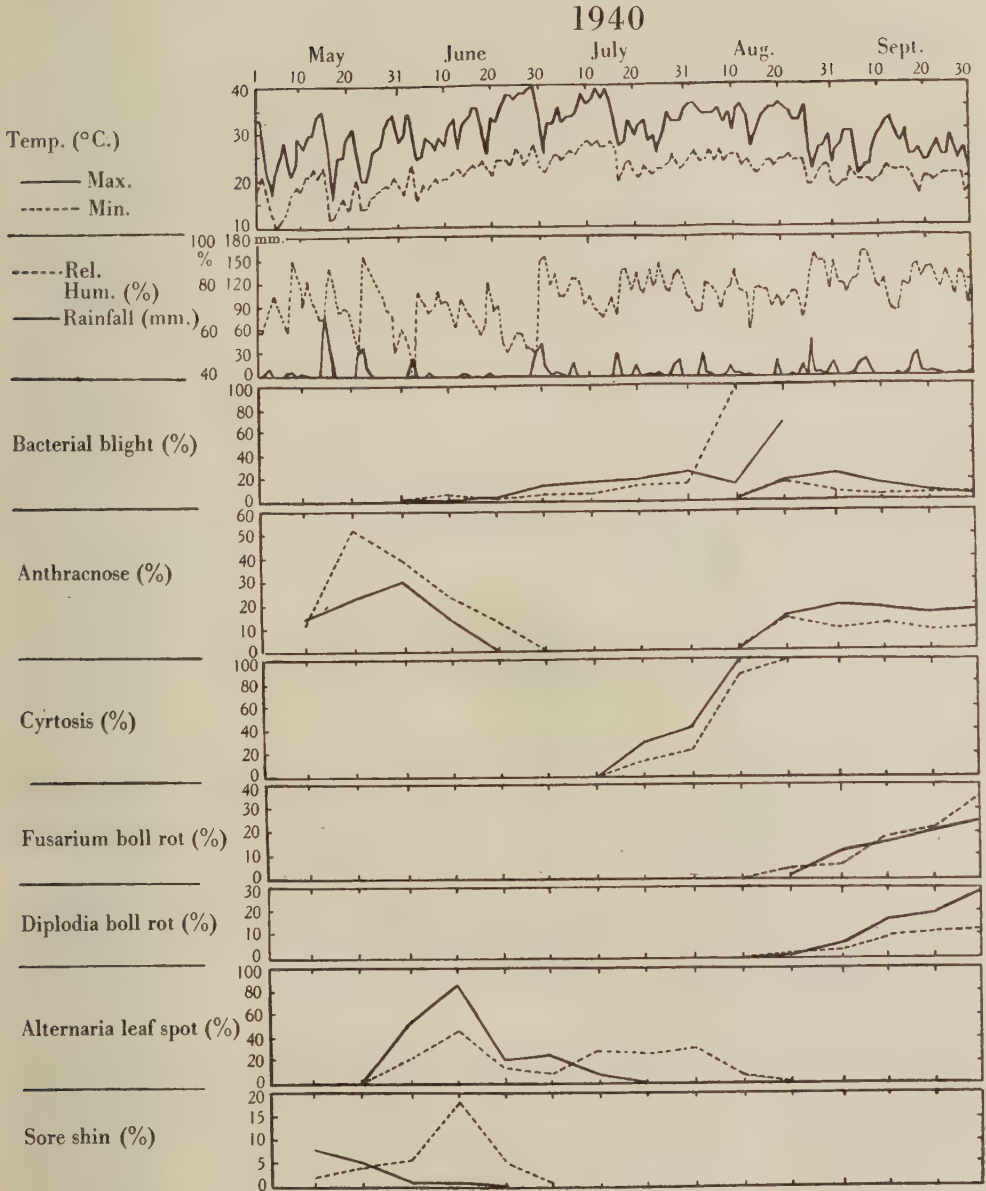


Fig. 3. Graphic summary of daily meteorological records and the development of common diseases of cotton. Suinin, Szechuan Province, China, 1940. — Delfos 531. - - - Local var.

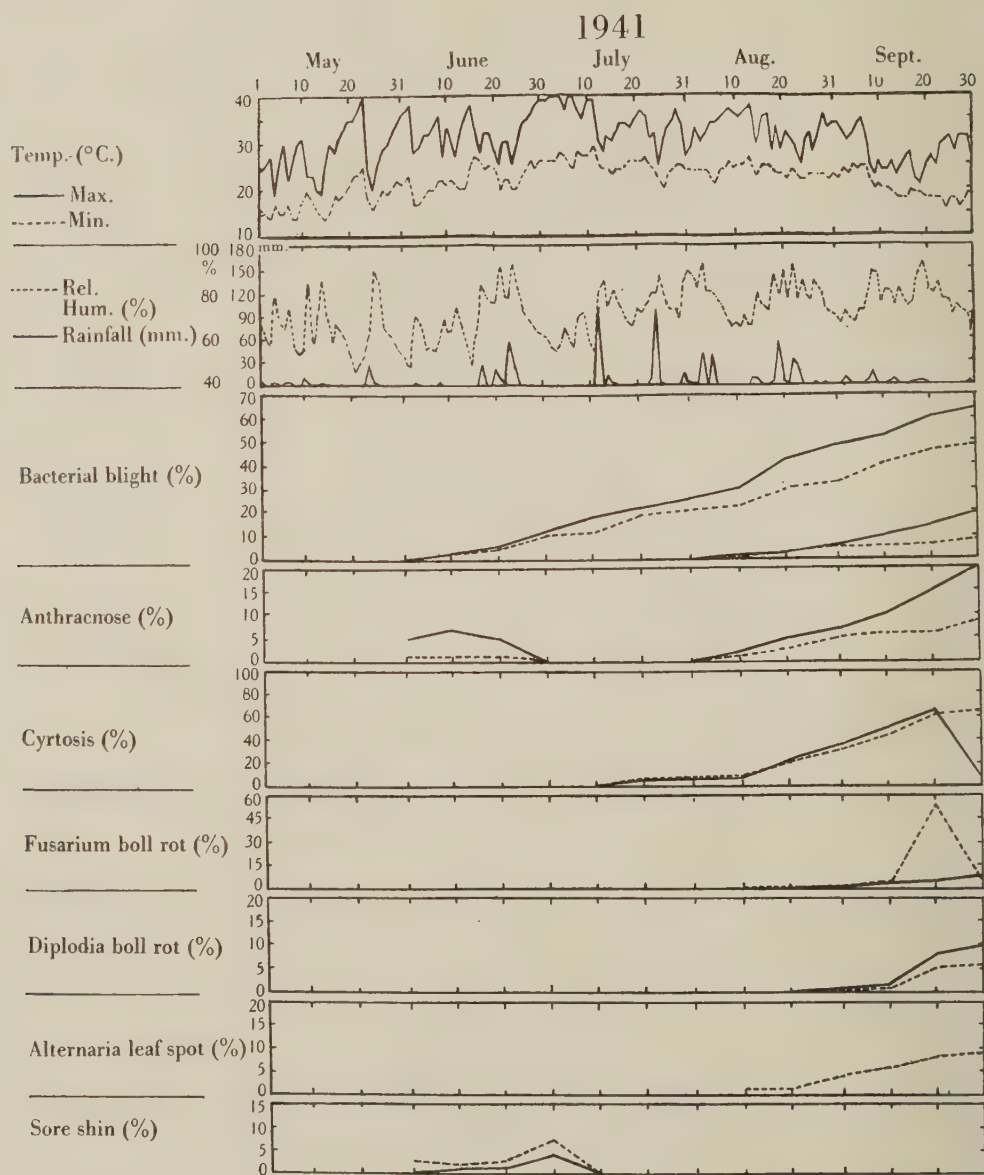


Fig. 4. Graphic summary of daily meteorological records and the development of common diseases of cotton. Suinin, Szechuan Province, China, 1941. — Delfos 531. - - - Local var.

ture, though important in determining the initiation of diseases and epidemics in many cases, appears to be subservient to the influence of air humidity under local conditions. This is because, first, the variation of temperature between ordinary years is relatively too small to account for the remarkable difference in disease situation; and secondly, the mean daily temperature during the growing season of cotton is usually within the range of 20–30°C., which includes the optima for the growth of the pathogens of most cotton diseases. As shown by previous investigators,

distribution rather than the amount of rainfall that count in effecting the initiation of diseases.

Based on the relative humidity alone, the occurrence and prevalence of a number of common diseases of cotton can be satisfactorily interpreted. Relative humidity was low in May 1938, but became higher than in all other years from June to the time of picking, except for a brief period in July and August. Accordingly, the seedling diseases were inconsequential, while the diseases affecting the mature plants, including the boll rots, were serious. In 1939 the condition was approximately the converse of the preceding year. A high humidity encouraged the development of seedling diseases, while most diseases were greatly checked by low humidity in the later part of the growing season. In the boll stage, a very dry condition eliminated almost entirely the boll rots. During this and the preceding years, leaf infection of anthracnose was especially prevalent in local varieties of cotton as a result of high humidity in June, but this did not occur in the other 2 years. This phase of infection is not a serious manifestation in upland cotton, but often damages the Asiatic varieties by causing premature falling of leaves and even the death of the whole plants.

In 1940 the humidity in May was the highest of the 4 years concerned, the seedlings consequently suffered from heavy infection of anthracnose, but the incidence of bacterial blight was almost negligible. Both diseases are known to be carried over the winter mainly with the seeds. The scarcity of both lesions on cotton bolls in 1939 would be expected to result in a reduction in seedling infection in 1940. However, since this was true only of bacterial blight, the abundance of anthracnose as a seedling parasite in 1940 must have originated from a different source from the seed infection through boll rot. The anthracnose fungus was shown by Converse (1919), Edgerton (1912) and Weindling *et al.* (1941) to be able to survive a considerable length of time as a saprophyte on dead or apparently healthy portions of stem and leaves of cotton. Weindling *et al.* (1941) further demonstrated that spores of the fungus are distributed within seed lots and perhaps to other lots by mixing of seed and trash—hulls, unbroken locks, and other debris—during the ginning process. This is thought to account for the considerable amount of *Glomerella* on seed obtained from fields in the southern United States in which very little or no anthracnose boll rot was apparent. Observations proved all this to be true under our local conditions. During its saprophytic life, the fungus has many chances to contaminate the seeds when still in open bolls through the aid of rain water and later during the ginning process. The causal organism of bacterial blight, on the other hand, may not have equal ability to perpetuate itself in this way. *Alternaria* leaf spot was also highly destructive in this stage, but the causal fungus is known to possess spores with higher

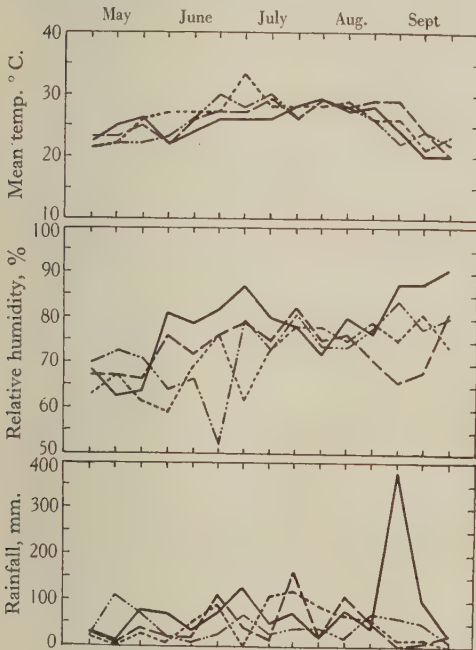


Fig. 5. Graphic summary of averages of air temperature and relative humidity, and totals of rainfall of 10-day periods. Suinin, Szechuan Province, China, 1938–41.

— 1938 - - - 1939
- - - 1940 - · - 1941

most diseases of summer crops are favoured by a combination of high temperature and high humidity. High humidity, however, is usually associated with low temperature. In comparing the variation of temperature within the range of 20–30°C. with the variation of humidity within the range of 50–90%, the latter would naturally be more influential. Rainfall, besides its influence on the temperature, air humidity and soil moisture, is important in itself as a dissemination agent for a large number of fungal and bacterial pathogens. To accomplish the latter, the occurrence of frequent driving rains is essential, but not heavy downpours. It is the frequency and

resistance to desiccation and might have other means of overwintering than contamination or infection of seeds.

In July of the same year it was unusually dry, which prevented the development of leaf and stem diseases, such as bacterial blight, *Alternaria* leaf spot, and anthracnose. A gradual increase in humidity in late August and September brought about a high incidence of bacterial blight and severe outbreak of boll rots, which were comparable to those of 1938 in their severity. This offers evidence that heavy rainfalls occurring at the end of August and early September in 1938 failed to increase materially the incidence of diseases.

In 1941 the humidity was low before the middle of July. After that date the rise of humidity resulted in a gradual increase of bacterial blight in late season of growth and an outbreak of various boll rots. *Alternaria* leaf spot also appeared in a much later stage than normal.

Sore shin, as a soil-borne disease, is chiefly influenced by soil moisture and soil temperature in its occurrence, while air humidity may determine its intensity of infection during the post-emerging stage. A prolonged dry period before and after the sowing, as happened in 1938 and 1941, greatly reduced its incidence. Ordinarily the disease appears in May soon after the emergence of seedlings, and the rise of temperature in a later period prevents its prevalence. In 1940, however, the low soil temperature in May (21.2°C. average and approximately 2°C. lower than the normal) permitted the occurrence of a high percentage of infection as late as early June.

Cyrtosis, as a leafhopper injury, responded to the climatic factors differently from the diseases of fungal and bacterial origin. Cook (1920) noted that the disorder developed early in hot weather in the spring and was less prevalent in shady places. Teng (1936) also demonstrated that the life cycle of the insect was completed in a shorter period at higher temperatures. Observations indicated that in this region the rapid reproduction of the insect begins ordinarily in July. The occurrence of high temperature in late June or in July, as encountered in 1940 and 1941, hastened the hatching and moulting of the leafhopper and hence the appearance of the disease. Should heavy rains occur during the period of rapid reproduction of the insect, as happened in 1939, to check its breeding, the incidence of the disease would be reduced accordingly.

EFFECT OF TIME OF SOWING

Change in the time of sowing may indirectly influence the development of certain diseases due to the differences in the environmental conditions throughout the growing season of the host. The results may be compared with those obtained from observation on the seasonal development of such diseases.

From 1938 to 1940 sowings of cotton seed were made throughout March to late May or early June at intervals of 1 or 2 weeks. In 1939 only an upland variety, Delfos, was used, while in two other years both the upland cotton, Trice for 1938 and Delfos for 1940, and Asiatic cotton were included. In Suinin, where the experiments were made, the soil temperature at seed level, i.e. 2 in. below the surface, averaged about 13°C. in March, 17°C. in April, 23°C. in May, and 25°C. in June. With the increase of soil temperature, the length of time required for seedling emergence was reduced from 11-18 days in

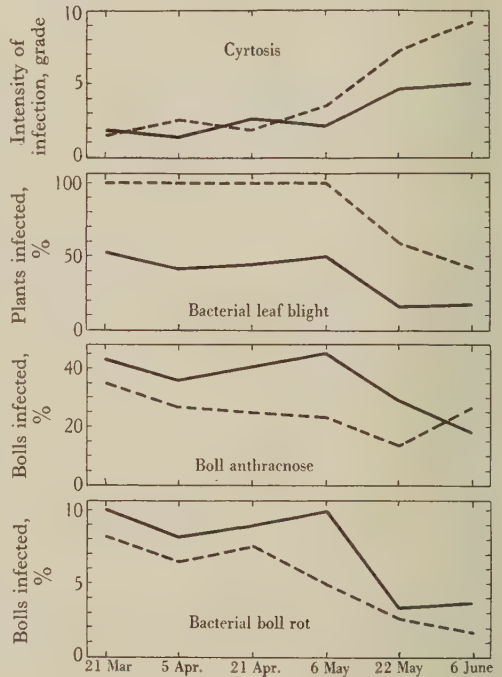


Fig. 6. Effect of time of sowing on the development of cyrtosis, bacterial blight and anthracnose of cotton.

----- Local var. ——— Trice

March to 7-12 days in April and 5-8 days in May and June. Since supply of moisture and oxygen are both required, a very dry or very wet condition in the soil decreases the rate of germination. Several important diseases, affecting either the seedlings or the mature plants, were recorded. The results, which are summarized in Fig. 6 and Table 1, agree fairly well for the two species of cotton used.

Seedling diseases. The incidence of anthracnose and sore shin on seedlings was recorded for different sowings in three successive years, 1938-40 inclusive (Table 1). Both diseases are frequently responsible for poor stand, but pre-emergence killing is rarely

due to anthracnose. Under conditions favourable for the growth of cotton, the seedlings infected are often capable of sufficient recovery to assume normal growth.

The causal fungus of anthracnose is essentially seed-borne, and internally infected seeds are less influenced by environmental conditions than are those externally contaminated. Results obtained show a definite correlation between the prevalence of the disease and air humidity. A dry atmosphere appears to check the manifestation and extension of the lesions and favours the recovery of the seedlings. Moreover, moisture is necessary for the fructification

The causal fungus of the sore shin disease differs from that of anthracnose in being a facultative parasite surviving in the soil. Therefore its spread, propagation, and activity depend chiefly upon a favourable condition of the soil. Among the conditions considered, soil moisture appears to be the most important. Unfortunately records of soil moisture during the experimental period are not available, but the data on the distribution and amount of precipitation may, perhaps, indicate the moisture condition of the soil.

In 1938, following an extraordinarily dry winter, the rainfall in the spring was hardly sufficient to

TABLE 1. *Effect of date of sowing on the development of seedling diseases of cotton*

Date of sowing	Relative humidity* %	Upland cotton			Asiatic cotton		
		Seedlings emerged %	Seedlings infected by		Seedlings emerged %	Seedlings infected by	
			Sore shin %	Anthracnose %		Sore shin %	Anthracnose %
1938 21 Mar.	76.3	53	0.7	26.1	55	2.0	31.5
5 Apr.	72.5	84	3.1	41.1	97	0.8	50.2
21 "	67.6	63	1.0	15.4	66	1.0	8.6
6 May	63.7	82	3.3	16.7	94	4.0	2.3
22 "	82.7	84	0.8	74.8	88	2.6	55.2
6 June	82.0	91	0.2	46.3	89	0.6	29.6
1939 14 Mar.	73.3	35	1.7	39.3	—	—	—
21 "	77.0	87	1.2	58.6	—	—	—
30 "	84.2	58	0.2	53.9	—	—	—
6 Apr.	80.5	54	1.2	26.8	—	—	—
14 "	71.9	94	2.0	14.9	—	—	—
21 "	67.0	96	3.9	7.8	—	—	—
29 "	65.9	95	13.3	35.6	—	—	—
6 May	66.4	89	4.8	17.5	—	—	—
13 "	64.0	89	3.4	14.4	—	—	—
1940 21 Mar.	65.1	86	2.8	16.8	75	1.0	4.1
30 "	60.3	57	1.1	24.9	61	1.2	20.0
6 Apr.	58.2	54	1.7	11.0	76	1.1	4.7
14 "	62.9	93	0.8	13.7	87	2.1	10.8
21 "	72.3	96	2.8	60.7	96	3.5	78.5
29 "	74.3	95	6.5	72.6	96	3.4	77.2
6 May	74.2	89	4.0	49.9	94	8.4	45.0
13 "	73.4	90	12.3	45.3	84	18.2	40.6

* Average of a period of 10 days following the emergence of seedlings.

and the spread of the fungus as well as the secondary infection of the seedlings. As shown in Table 1, a prolonged dry period with an average humidity lower than 70% after the emergence of seedlings usually results in a low percentage of infection. The only case where the statement did not hold true is in the lot sown on 29 Apr. 1939 in which high infection occurred after a period of fairly low humidity. The present results appear to agree with the general belief that cold and wet weather predisposes the seedlings to infection by anthracnose. High humidity is usually accompanied by a relatively low temperature, and probably the two factors interact to influence the severity of the disease.

moisten the soil. Therefore, the sore shin disease was checked to an inconsequential degree throughout all the sowings. In 1939, although the preceding winter and the early spring were also dry, in the period from April to early May the soil moisture was gradually increased by frequent and well-distributed rainfalls. In April, a total of 21 rainy days in addition to 8 cloudy days were recorded, and in May 6 out of the first 10 days were rainy. The accumulation of moisture in the soil gradually activated the fungus and eventually brought about an increasing incidence of the disease in the sowings of April, with the attainment of the highest infection in the last sowing. From 11 to 27 May was a dry period, with a total

precipitation of 7.2 mm. only. In accordance with this condition, the incidence of sore shin declined again in the two May sowings. In 1940, the occurrence of sore shin was parallel to that of anthracnose. The four earlier sowings were made during a dry period from 21 Mar. to 25 Apr. with only 28.1 mm. of precipitation. On 26 Apr., soil moisture was brought up by a heavy rain, resulting in a higher infection in the sowing made 5 days before. During May, before the 24th was a very wet period with a precipitation of 210.8 mm. in all: the high soil moisture caused a considerable increase of the disease.

Walker (1928) presented experimental results to demonstrate that the sore shin disease was favoured by a low soil temperature of 17–23°C., but was not apparently affected by soil moisture. The present results do not agree with this statement. In his experiments, the fungus was thoroughly incorporated into the sterilized soil which was allowed to stand for a period before planting to permit the fungus to make good growth. In short, the inoculum was abundantly provided and well distributed already in the soil before the suspect came into contact with it. In nature, however, the situation is entirely different: the fungus survives in the soil in competition with numerous micro-organisms, and it has to wait for a favourable condition to start active growth before an epidemic can be produced. In this case, soil moisture would play an important role in influencing its activity and hence the occurrence of the disease. Shen & Chow (1937) also correlated the severity of sore shin in different localities with the amount of precipitation in spring. Local soil temperature from March to May seldom exceeded 23°C., which Walker (1928) set as the upper limit of optimal range for the fatal effect of the disease. In certain cases, however, it is still a factor limiting the late occurrence of the disease. The serious outbreak of sore shin in late sowings in 1940 is due to the especially low soil temperature in May, which, as mentioned already, was approximately 2°C. lower in average than the normal.

Mature plant diseases. Certain diseases affecting the cotton plant at the mature stage were also considerably influenced by the time of sowing. The results of 1938 (Fig. 6) show that the two late sowings, one on 22 May and the other on 6 June, had heavier infection of cyrtosis but lower incidence of bacterial blight and anthracnose than earlier ones. The former case is in agreement with the observation of Cook (1920). As cyrtosis is of insect origin, late sowing would expose the cotton plants to a heavier infestation owing to the larger population of the leafhopper at a later period. For the diseases of fungal and bacterial origin, late sowings would postpone maturity and enable the host plants to escape the rainy period during late season of growth so as to reduce the chances of being infected, but the growth of the

cotton plants is decidedly inferior than those sown earlier.

EFFECT OF CULTURAL PRACTICES

Modifications of several cultural practices were tested for their influence on the development of certain diseases of cotton: such changing of the immediate surroundings of plants has not so profound an influence as a change in climatic conditions. All the experiments were arranged in randomized blocks, with at least four replications. A local variety of the Asiatic cotton was used throughout.

Fertilizers. Among the plant nutrients, nitrogen is known to influence most profoundly the prevalence of plant diseases through its effect on the host growth. In the experiments, three different kinds of nitrogenous fertilizers, namely, ammonium sulphate, cotton-seed cake, and rape-seed cake, were applied, in addition to the manure, in either single or double dosages. The rate of application of all the fertilizers was approximately equal in the amount of nitrogen contained. Plots with no fertilizer or with the manure applied alone served as the checks. Seedling diseases, including anthracnose and sore shin, were found not to be significantly affected. The prevalence of stem, leaf, and boll diseases in each treatment are summarized in Table 2. In general, all the nitrogenous fertilizers tested caused an increase in the incidence of the diseases. Ammonium sulphate, especially when applied in double dosages, exerted a greater influence than the two others, due apparently to the fact that it is more readily available to the plants. Among the diseases recorded, anthracnose, bacterial blight, and areolate mildew were more clearly affected, while cyrtosis appeared to be little influenced.

Another experiment was carried out to ascertain the relative effect of the three essential elements, nitrogen, phosphorus and potassium. The fertilizer treatment consisted of a single element, combination of two elements and also of all three elements at the rate of 200 lb. of ammonium sulphate, 250 lb. of superphosphate and 27 lb. of potassium sulphate per acre, equivalent to 40 lb. of nitrogen, 40 lb. of phosphorus, and 13.5 lb. of potassium. The results failed to give any significant difference except when nitrogen fertilizer was applied either alone or in combination with other elements. In that case, anthracnose was increased though the yield of cotton was also higher.

Density of stand. Effect of various spacing and density of stands adjusted at the time of thinning, both in hills and in rows, were tested. The results as summarized in Table 3 indicated the general tendency to be that the denser the plant stand in each hill or the closer the spacing in drilling, the higher the infection by boll diseases, although the opposite is true with stem and leaf diseases. The latter situation is in contradiction with the general expectation. It is generally believed that a denser stand would

bring about a condition of higher humidity and poorer aeration, thus facilitating the spread and development of most plant diseases. Hansford (1936)

effect against the spread of the leafhoppers. Consequently this particular crop exerted very little effect in reducing the disease.

TABLE 2. *Effect of nitrogenous fertilizers on the development of cotton diseases*

Treatment	Estimated quantity of N added (lb./acre)	Stem and leaf diseases			Boll diseases		
		Bacterial blight %	Areolate mildew %	Cyrtosis Grade*	Anthrax-nose %	Bacterial blight %	Fusarium rot %
None	0	57.9	9.9	2.0	4.3	3.0	0.5
Manure alone	26.8	72.1	16.8	1.5	6.3	4.7	1.0
Ammonium sulphate	53.5	70.2	18.4	1.5	4.1	3.8	0.9
+manure	80.3	85.5	29.1	2.6	9.5	5.1	1.7
Cotton-seed cake	53.5	79.9	24.1	1.3	8.9	4.1	1.0
+manure	80.3	90.2	26.4	1.4	8.0	4.2	0.8
Rape-seed cake	53.5	85.2	23.3	1.5	6.5	3.8	0.9
+manure	80.3	87.4	29.9	1.6	8.3	4.3	1.0

* The grading is based on a severity scale of 0-10, the 10 being the greatest severity of cyrtosis and the 0 healthy (Shen & Chow, 1937).

TABLE 3. *Effect of spacing and density of stands on the development of cotton diseases*

Method of sowing	Spacing between hills or plants in.	Plants in each hill No.	Stem and leaf diseases			Boll diseases	
			Bacterial blight %	Areolate mildew %	Cyrtosis Grade*	Bacterial blight %	Anthraxnose %
In hill	6	2	88.9	81.6	0.8	16.9	7.1
	12	1	99.5	93.2	2.5	10.9	5.3
	12	2	97.5	90.8	1.5	10.5	5.4
	12	3	97.4	89.8	1.1	11.3	5.5
	12	5	91.7	85.6	0.7	12.1	5.8
In drill	4.5	—	89.2	88.2	0.9	12.7	9.0
	2.5	—	90.0	83.5	0.8	16.8	7.1

* Grading (see Table 2).

reported increase in intensity of bacterial blight of cotton by close spacing. The present results, therefore, need further verification.

Mixed cropping system. Mixed cropping system is generally practised by farmers in the cotton district of this region for the insuring of return in case of the failure of cotton in certain years. Four common summer crops, soybean, peanut, corn, and sesame, were selected and interplanted separately with cotton in various spacings: plots with cotton alone served as the check. The results obtained failed to indicate any definite relation between the mixed cropping and the incidence of all the diseases under observation except cyrtosis. By interplanting with a crop not attacked by the hoppers, the spread of the insect was somewhat delayed. This is indicated by the results in Table 4; as the number of rows of cotton between the intercrops decreased, the intensity of infection also declined. Among the four mixing crops tested, soybean was reported (Teng, 1936) to be susceptible to cyrtosis and hence had no defensive

TABLE 4. *Effect of mixed cropping on the development of cyrtosis of cotton*

Crop mixed with cotton	Rows of cotton between intercrop No.	Severity of cyrtosis Grade*
Peanut	2	1.9
	4	2.1
	6	2.7
	Check	2.5
Soybean	2	1.4
	4	1.5
	6	1.7
	Check	1.9
Corn	2	0.7
	4	1.2
	6	1.1
	Check	1.6
Sesame	2	0.8
	4	1.6
	6	1.7
	Check	1.9

* Grading (see Table 2).

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Diseases of the gladiolus

I. Control of hard rot, due to *Septoria gladioli* Passer., by fungicidal treatment of the corms

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(With 1 Text-figure)

Losses from hard rot, measured by an arbitrary disease index, were reduced by treating the dehusked corms before planting with mercuric chloride (with or without the addition of 1.0% hydrochloric acid), mercurous chloride (calomel), three proprietary mercury compounds (Aretan, Uspulun and Ceresan) and one proprietary non-mercury compound (Folosan). Calomel was the least effective. All the treatments were relatively less effective when corms with definite lesions were treated.

The weight of clean corms produced per old corm planted (weight index) was usually increased by all the fungicides tried, but calomel and Ceresan were less satisfactory than the others.

Mercuric chloride (3 hr. steep in a 0.1% solution) was not rendered more effective by the addition of hydrochloric acid nor by a preliminary dip in methylated spirits to facilitate wetting, while the addition of a proprietary wetting compound (Agral) was definitely harmful to the corms and usually less effective than mercuric chloride alone. Increase in time of steeping or concentration of mercuric chloride was not beneficial and was sometimes harmful. Reduction in time of steeping to 1 hr. gave promising results.

Treatment in November had some advantages over treatment in March.

All the mercury compounds tended to delay flowering, this being most marked in the presence of the wetting compound. Stunted foliage and poor quality flowers resulted from the use of Ceresan.

INTRODUCTORY

Hard rot, caused by *Septoria gladioli* Passer., is a serious disease of gladiolus corms in England and elsewhere. A detailed description of the symptoms and a summary of the literature have been given by Moore (1939). Massey (1916) considered that infection of clean corms took place from soil where a diseased crop had been grown and that spores produced on the leaves were washed down and infected

the young corms. Accordingly, most investigators have recommended control by planting in clean soil and spraying the foliage with Bordeaux mixture. Moore (1939) stated that this disease was then the most common one on corms imported into this country. Such diseased stocks planted in soil where gladioli had not previously been grown at the Imperial College of Science Field Station at Slough have invariably yielded a high proportion of diseased corms. Thus, a corm treatment designed to prevent

transmission of the disease from old to new corms is desirable. Massey (1916) was unable to control the disease by steeping the corms for 18 hr. in formalin or 1/1000 mercuric chloride or by rolling them in sulphur, slaked lime, acid phosphate or soot. White (1928) recommended the use of a 0.1% solution of mercuric chloride with the addition of 1.0% hydrochloric acid. Martin (1930) reported some control from a similar treatment for 7 hr., but the corms afterwards showed damage. Similar control but without damage was obtained by the use of 6-ethyl mercury chloride. Löbner (1925) obtained good results from steeping the corms for 2 hr. in a 0.25% solution of Uspulun, the use of which was also recommended by Pape (1925) and Riefenstahl (1934), while Drayton (1929) obtained satisfactory results by dipping the corms for 15 min. in a 5.0% solution of Semesan or Uspulun at 50° C. Treatment at planting time with 1/1000 mercuric chloride (30 min. to 2 hr.), calomel or organic mercury compounds was recommended by McCulloch & Weigel (1941).

A single experiment carried out in this laboratory by Burrows (unpublished data) gave good control from steeping the corms for 3 hr. in a 0.1 or 0.25% solution of mercuric chloride, but a 0.5% solution was less satisfactory. In a single small-scale experiment he obtained a reduction in the amount of disease to less than half that in the controls by steeping the corms for 3 hr. in a 0.4% solution of Uspulun. Dusting with Folosan slightly reduced the amount of disease, but a combination of the two treatments gave no improvement on Uspulun alone. Treatment with formalin actually increased the amount of disease occurring among the new corms probably as a result of penetration by the fungus following chemical injury to the old corms. Bray (1942) showed that certain copper compounds were also harmful.

The writer has investigated the relative efficiency of certain mercury compounds and of Folosan and of different methods of applying these in experiments with infected stocks during four growing seasons.

GENERAL EXPERIMENTAL METHODS

Corms were lifted in October and the shoots were cut off about 2 in. above the corm. The cause and extent of disease lesions could not usually be determined unless the outer husks (leaf bases) were removed. Discoloured patches on these husks bore no relation to the lesions on the corm surface except when the disease was so severe that the corm would normally be discarded. Extensive hard rot, dry rot or scab lesions were often present on corms which looked sound when the husks were intact. Similar observations were made by Massey with hard rot (1916) and dry rot (1928). Fungicidal treatment was unlikely to be effective unless the husks were removed, since these would prevent the fungicide from coming into contact with the lesions on the corm

surface. Preliminary experiments by Burrows (unpublished data) and the writer indicated that dehusking did not render the corms more liable to hard rot nor injure them in any way. Accordingly, after drying for 1-2 weeks the corms were cleaned (i.e. the shrivelled parent corms, roots, cormels and husks were removed), examined for disease lesions and stored in trays in an unheated shed. The trays were covered with sacks or straw in frosty weather. Fungicidal treatments were given in March, or occasionally in the autumn, and the corms were planted in March or April in plots in which gladioli had not previously been grown. The treatments were usually replicated and the corms were planted in randomized blocks. The experimental unit was usually twenty corms. Unreplicated experiments were also performed with small lots of corms. Corms failing to grow were lifted after a few weeks. During the summer plants with prematurely brown foliage were also removed. The causes of these failures were determined by observation or isolation. Flowers were allowed to open in order to permit observations of quality and date of flowering, but were then cut off above the first leaf to prevent loss of corm weight through seeding.

The following arbitrary methods of estimating the amount of disease were adopted: (1) *Disease index*. Old corms failing to develop through *Septoria* injury or plants dying from this cause during the growing season were awarded 100 points. New corms showing hard-rot lesions when lifted and cleaned were rated as follows: severely diseased (i.e. obviously so badly diseased at neck or base as to prevent growth) 50 points, moderately diseased (i.e. numerous lesions but not involving main bud or root initials) 30 points, slightly diseased (i.e. few small scattered lesions) 10 points. Lesions other than those of hard rot were ignored. The total points were then divided by the number of old corms planted, less any which failed from other causes. (2) *Weight index*. The weight of sound corms harvested was divided by the number of old corms planted, less those failing from other causes.

In large-scale experiments differences between treated lots as shown by disease index and weight index were tested for significance by the usual statistical methods.

Corm treatments with various fungicides. A large stock of var. War infected with *Septoria*, smaller stocks of vars. Wolfgang von Goethe, Ave Maria and Flaming Sword and small lots of other varieties similarly infected, a large stock of var. Rose Precose infected not only with *Septoria* but also with *Sclerotinia gladioli*, *Bacterium marginatum* and *Botrytis* sp., a large stock of var. Lilac Wonder practically free from disease, smaller healthy stocks and a few stocks infected with *Sclerotinia* were available. These were given the following treatments: (1) no treatment (none); (2) 3 hr. in a 0.1% solution mercuric chloride

(HgCl_2); (3) as (2) but with 1.0% hydrochloric acid added to the solution ($\text{HgCl}_2 + \text{HCl}$); (4) 3 hr. in a 0.5% solution of the proprietary mercury compound Aretan (Aretan, cold); (5) 15 min., or in later experiments 1 hr., in a similar solution at 42° C. (Aretan, warm); (6) and (7) similar treatment to that of (5) and (6) with the proprietary mercury compound Uspulun (Uspulun, cold and Uspulun, warm); (8) 5 min. in a 1.0% suspension of mercurous chloride, the suspension being stirred all the time (calomel); (9) shaken up in a paper bag with the proprietary mercury compound Ceresan and then

slightly diseased corms from infected stocks are given in Table 1 and those with more severely diseased corms in Table 2. Disease index values (Table 1) show that any of the treatments when applied to clean or slightly diseased corms, reduces the amount of disease developing during the growing season. All the mercury compounds, with the exception of calomel, were about equally effective. The inferiority of calomel was proved to be significant. The addition of hydrochloric acid did not increase the efficiency of mercuric chloride. In a number of small-scale experiments, not included in Tables 1 and 2, Uspulun

TABLE 1. *Fungicidal treatment of clean or slightly diseased corms from infected stocks*

Variety	No. of corms per treatment	Index	Treatment							
			None	HgCl_2	$\text{HgCl}_2 + \text{HCl}$	Aretan warm	Aretan cold	Calomel	Ceresan	Folosan
War	6 × 20	D.I.	16.0	2.7	—	2.6	—	4.7	—	—
"	6 × 20	W.I.	25.9	35.0	—	32.0	—	35.2	—	—
"	5 × 20	D.I.	9.5	1.3	2.1	0.2*	1.5	—	0.2	2.6
"	5 × 20	W.I.	33.2	49.9	47.6	47.7	46.1	—	44.2	50.0
"	4 × 20	D.I.	8.9	0.8	—	—	—	3.3	—	—
"	4 × 20	W.I.	42.7	52.0	—	—	—	45.1	—	—
"	20	D.I.	2.5	0.0	—	0.0*	0.0	—	—	1.0
"	20	W.I.	54.1	67.9	—	77.0	67.5	—	—	61.6
Wolfgang von Goethe	4 × 20	D.I.	16.5	8.5	—	0.4	—	—	—	—
"	4 × 20	W.I.	38.9	33.5	—	43.4	—	—	—	—
"	3 × 20	D.I.	29.1	3.7	—	—	—	10.0	—	—
"	3 × 20	W.I.	30.9	45.8	—	—	—	39.5	—	—
"	20	D.I.	8.5	0.0	0.0	—	—	—	—	2.0
"	20	W.I.	51.0	90.0	68.5	—	—	—	—	87.0
Flaming Sword	3 × 10	D.I.	11.3	2.2	—	—	—	—	—	—
"	3 × 10	W.I.	29.7	31.7	—	—	—	—	—	—
"	2 × 25	D.I.	16.0	4.1	—	2.1	—	—	—	—
"	2 × 25	W.I.	17.4	19.9	—	24.8	—	—	—	—
"	25	D.I.	8.0	—	—	—	—	—	0.0	0.0
"	25	W.I.	22.5	—	—	—	—	—	22.5	23.4
Rose Precose	4 × 20	D.I.	12.0	7.7	—	8.5	—	7.5	—	—
"	4 × 20	W.I.	40.1	44.7	—	40.7	—	47.9	—	—
"	4 × 20	D.I.	5.1	1.7	—	4.2	1.5	—	1.1	2.0
"	4 × 20	W.I.	40.0	40.8	—	32.8	44.8	—	26.9	50.0
Ave Maria	4 × 20	D.I.	14.4	2.3	—	5.1	—	—	—	—
"	4 × 20	W.I.	20.1	25.4	—	24.8	—	—	—	—
"	3 × 20	D.I.	13.9	10.9	—	—	—	—	—	—
"	3 × 20	W.I.	28.6	30.4	—	—	—	—	—	—

* Treated 15 min., rest treated 1 hr.

D.I. = disease index, W.I. = weight index.

shaken free of excess powder (Ceresan); (10) dipped in water and rolled in the proprietary non-mercury fungicide Folosan (Folosan). No one stock received all these treatments at the same time, and with some of the small lots only one or two treatments could be tested. A small-scale pot experiment indicated that Aretan was superior to Uspulun and that warm Aretan was superior to cold (particularly in its effects on other diseases to be considered in a later paper). Moreover 1 hr. in warm Aretan was greatly superior to 15 min. This experiment was used as a guide in designing the larger scale trials.

The results obtained with apparently healthy or

did not compare favourably with Aretan, so that, owing to the greater cost and higher mercury content of the former it was omitted from the large-scale trials. Folosan usually gave less satisfactory results than the mercury fungicides, but this difference was not significant in the only two large-scale tests made. The results measured by weight index were in general similar but Ceresan gave a low weight of clean corms produced, sometimes below that of the untreated controls (owing to chemical injury which was also clearly seen in the stunted foliage and poor flowers), while Folosan gave the greatest weight of clean corms, probably through the favourable effect of this

fungicide on *Botrytis* attack which will be considered in a later paper. These results are further emphasized in Table 3, which compares treatments in pairs including only those experiments large enough to enable tests of significance to be made.

When corms with definite hard-rot lesions were treated the effect of fungicides as measured by disease index was much less marked, but the weight index showed improvement by the use of mercuric chloride or Aretan. Since only relatively small numbers of

TABLE 2. *Fungicidal treatment of corms showing hard rot lesions*

Variety	No. of corms per treatment	Index	Treatment					
			None	HgCl ₂	HgCl ₂ + HCl	Aretan warm	Aretan cold	Uspulun hot
War	2 × 25	D.I.	63.4	36.9	—	33.8	—	—
"	2 × 25	W.I.	3.4	14.7	—	6.0	—	—
"	20	D.I.	59.0	71.0	65.0	—	56.5	—
"	20	W.I.	9.0	17.5	18.5	—	21.0	—
"	20	D.I.	60.5	57.5	—	—	—	—
"	20	W.I.	15.0	11.6	—	—	—	—
Wolfgang von Goethe	2 × 20	D.I.	19.8	9.7	5.8	—	—	—
"	2 × 20	W.I.	26.5	39.7	38.3	—	—	—
"	20	D.I.	33.0	26.5	—	11.0	—	—
"	20	W.I.	16.5	18.0	—	23.0	—	—
Rose Precose	3 × 20	D.I.	28.6	21.0	—	—	—	—
"	3 × 20	W.I.	31.8	38.0	—	—	—	—
Ave Maria	25	D.I.	36.4	16.4	—	15.7	—	—
"	25	W.I.	4.0	11.2	—	11.8	—	—
Lilac Wonder	25	D.I.	22.0	15.4	—	15.8	—	—
"	25	W.I.	30.4	34.6	—	30.8	—	—
"	20	D.I.	19.5	6.5	—	7.0	—	—
"	20	W.I.	21.0	26.0	—	37.0	—	—
*Wolfgang von Goethe	12	D.I.	12.3	8.5	—	7.5 (15 min.) 1.5 (1 hr.)	—	15.5 (15 min.) 5.0 (1 hr.)

* Pot experiment.

D.I. = disease index, W.I. = weight index.

TABLE 3. *Comparison of pairs of treatments*

Treatments compared	No. of trials	Disease index			Weight index		
		2nd treatment significantly better	No difference	2nd treatment significantly inferior	2nd treatment significantly better	No difference	2nd treatment significantly inferior
None v. 3 hr. 0.1 % HgCl ₂	11	11	0	0	5	6	0
None v. Aretan, cold	2	2	0	0	1	1	0
None v. Aretan, warm	6	5	1	0	4	2	0
None v. Calomel	4	4	0	0	3	1	1
None v. Ceresan	2	2	0	0	1	0	1
None v. Folosan	2	2	0	0	2	0	0
Calomel v. HgCl ₂	4	3	1	0	2	2	0
Aretan, warm v. HgCl ₂	6	1	4	1	1	4	1
Folosan v. HgCl ₂	2	1	1	0	0	1	1
HgCl ₂ + HCl v. HgCl ₂	1	0	1	0	0	1	0
16 hr. 0.1 % HgCl ₂ v. 3 hr.	2	2	0	0	1	1	0
6 hr. 0.1 % HgCl ₂ v. 3 hr.	1	0	1	0	0	1	0
1 hr. 0.1 % HgCl ₂ v. 3 hr.	2	0	2	0	0	2	0
3 hr. 0.25 % HgCl ₂ v. 0.1 %	3	0	3	0	2	1	0
3 hr. 0.1 % + Agral v. 0.1 %	3	2	1	0	2	1	0
3 hr. + Meths. v. 0.1 %	3	0	3	1	0	3	0
16 hr. 0.25 % HgCl ₂ v. 3 hr. 0.25 %	1	1	0	0	1	0	0

diseased corms were available replicated experiments were seldom possible and other fungicides were not tested.

Modifications in method of using mercuric chloride. Since a 3 hr. steep in 0.1 % mercuric chloride was at least as good as any of the other treatments tried, experiments were carried out to test the effect of increasing the concentration or the time of steeping and of more efficient wetting of the corms. In the small-scale pot experiment already referred to the results of increasing the time to 16 hr. or the concentration to 0.5 % were not encouraging. Burrows (unpublished data), however, obtained a slightly better control with 3 hr. in a 0.25 % solution than with 3 hr. in a 0.1 % solution. He also found that a 0.5 % solution was inferior, possibly owing to increased fungal attack following on chemical injury.

chloride alone in three replicated experiments but gave a greatly improved control in one small-scale experiment with severely diseased corms. The addition of a wetting compound (Agral) to the mercuric chloride solution gave more disease and less weight of clean corms compared with mercuric chloride alone in two out of three trials. The third trial was with a variety, Ave Maria, in which the corms are small and hard and which may therefore be particularly resistant to chemical injury. Fig. 1 shows that the higher disease index with Agral is due to a greater number of corms failing to develop or failing during the summer through hard-rot injury, and that the number of diseased new corms harvested is actually slightly less than those with mercuric chloride alone. This may be due to chemical injury of slightly diseased corms followed by increased activity of

TABLE 4. *Treatment of corms with mercuric chloride*

Variety	No. of corms per treatment	Index	Treatment										3 hr. 0.1 % + Agral	Dipped meths. 3 hr. 0.1 %
			None	3 hr. 0.5 %	16 hr. 0.25 %	3 hr. 0.25 %	16 hr. 0.1 %	6 hr. 0.1 %	3 hr. 0.1 %	1 hr. 0.1 %				
War	6 × 20	D.I.	16.0	—	—	—	—	—	2.7	0.3	6.0	—		
"	6 × 20	W.I.	25.9	—	—	—	—	—	35.0	34.3	24.5	—		
"	4 × 20	D.I.	8.9	—	3.0	0.8	2.0	0.3	0.8	—	—	—	0.1	
"	4 × 20	W.I.	42.7	—	41.7	45.8	42.2	50.9	52.0	—	—	—	52.0	
* "	95	D.I.	15.2	2.8	—	0.2	—	—	0.8	—	—	—	—	
"	95	W.I.	17.1	20.8	—	33.7	—	—	29.1	—	—	—	—	
War (diseased)	20	D.I.	60.5	—	—	41.1	66.0	—	57.5	—	—	—	37.5	
"	20	W.I.	15.0	—	—	2.6	1.0	—	11.6	—	—	—	26.5	
"	5 × 10	D.I.	63.4	—	—	—	—	—	36.9	—	49.1	—	—	
"	5 × 10	W.I.	3.4	—	—	—	—	—	14.7	—	7.6	—	—	
Ave Maria	4 × 20	D.I.	14.4	—	—	—	—	—	2.3	2.8	2.2	3.3		
"	4 × 20	W.I.	20.1	—	—	—	—	—	24.8	26.4	26.9	26.4		
"	3 × 20	D.I.	13.9	—	—	8.3	—	—	10.9	—	—	—	—	
"	3 × 20	W.I.	28.6	—	—	31.1	—	—	30.4	—	—	—	—	
Flaming Sword	3 × 10	D.I.	11.3	—	—	—	10.0	—	2.2	—	—	—	2.1	
"	3 × 10	W.I.	29.7	—	—	—	29.7	—	31.7	—	—	—	32.8	
† Wolfgang (diseased)	12	D.I.	12.3	10.0	—	—	10.0	—	8.5	—	—	—	—	

*Data from Burrows (unpublished work).

† Pot experiment.

The results of the present series of experiments are given in Table 4 and summarized in the lower half of Table 3. Both disease index and weight index show that an increase in length of steeping to 16 hr. is harmful compared with the 3 hr. treatment. This explains the unsatisfactory results obtained by Massey (1916) with mercuric chloride, since he steeped the corms for 18 hr. In a single large-scale experiment the effect of a 6 hr. steep was equivalent to that of a 3 hr. steep, while in two experiments a reduction of the time to 1 hr. did not reduce the efficiency of the mercuric chloride treatment of clean or slightly diseased corms. In treating severely diseased corms the longer steep might be more effective. The use of a preliminary dip in methylated spirits to facilitate wetting of the waxy surface of the corms showed no difference from mercuric

relatively deep-seated mycelium of *Septoria* or, more probably, to invasion by secondary parasites or soil saprophytes. This might even be an advantage if the object were to clean up a stock quickly, since the death of diseased corms leads to a smaller percentage of diseased new corms even though the weight of clean corms harvested is less than with mercuric chloride alone.

Effect of date of treatment. It is clear that none of the treatments completely killed the fungus in the diseased corms, and it is probable that the treatments acted through the more or less efficient prevention of spread of the mycelium from the lesions over the surface of the old corm to the young corms during the growing season. In the autumn the lesions are seen as water-soaked areas which extend only a little way into the corm, while by spring they have become

sunken and the fungus, while still not deep-seated, has penetrated a few millimetres. It was thought possible that treatment in autumn might succeed in destroying the fungus and would thus be superior to treatment in March. Table 5 gives the results of

experiments in which treatments with mercuric chloride or warm Aretan in November were compared with similar treatments in March. The experiments were complicated by the fact that the numbers rotting from storage rots (*Botrytis*, *Penicillium*, etc.) were greater in the control batches and in those set aside for treatment in March than in those treated in November. Thus the numbers planted varied, making statistical analysis impossible. Moreover, the losses from storage rot tended to occur chiefly among those corms already damaged by *Septoria*. With var. Wolfgang von Goethe which is particularly susceptible to *Botrytis* attack, this led to the elimination of the corms with hard-rot lesions in the control and March-treated lots and to a relatively high disease index in the November-treated lots. The weight index, however, was best in the latter. With var. War which is not greatly susceptible to *Botrytis* attack the November treatment was clearly superior to the March treatment.

Effect of treatments on growth and flowering. Corms treated with Ceresan produced stunted foliage and poor flowers, but no other treatment caused any loss of quality.

The number of shoots showing above the ground were counted at intervals of 2-3 days, and the average number of days from planting to emergence were calculated. Similarly, the numbers of flower spikes with at least one flower fully open were counted three times a week to give the average date of flowering. Since flowering extended over a long period, with a few blooms opening during the colder weather of September and October, it was thought advisable to check these results by the use of a second method. Accordingly, the average date by which 50% of the spikes showed at least one flower fully open was also determined. The results of fifteen replicated experiments (some of which were those included in Table 1 and some were with healthy stocks, no heavily diseased stocks being included) were tested for significance. All the treatments except Folsan, cold Aretan and 1 hr. in 0.1% mercuric chloride tended to delay flowering, but this delay was not always significant. Delay in emergence of shoots was less pronounced. Table 6 gives the number of experiments in which each treatment gave a significant retardation of flowering and emergence. The two

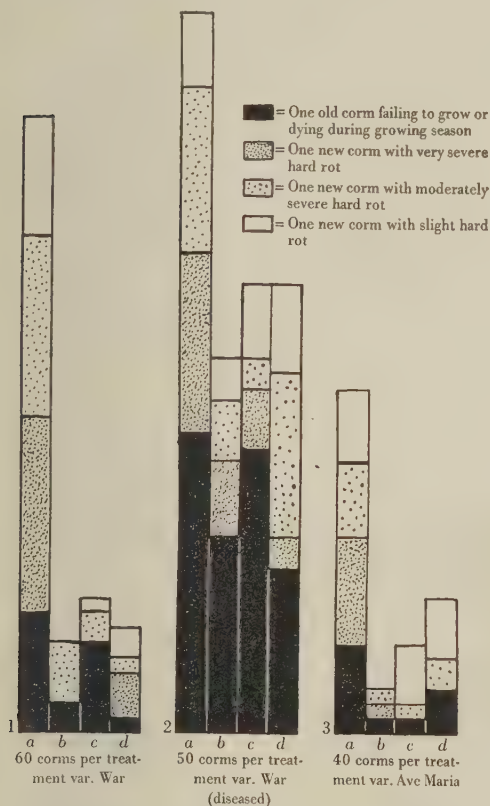


Fig. 1. Effect of fungicides on number of old corms failing from *Septoria* and number of new corms diseased. 1. Exp. 1, Table 4, var. War. 2. Exp. 5, Table 4, var. War, corms showing lesions when planted. 3. Exp. 6, Table 4, var. Ave Maria. Results with Aretan shown in Tables 1 and 2. Treatments: a=none, b=3 hr. 0.1% HgCl_2 , c=3 hr. 0.1% HgCl_2 + Agral, d=Aretan, warm.

TABLE 5. *Effect of date of treatment*

Variety	Original no. of corms per treatment	Index used	Treatment				
			None	HgCl_2 Nov.	HgCl_2 Mar.	Aretan warm Nov.	Aretan warm Mar.
War	100	D.I.	27.0	3.8	8.6	2.6	7.9
"	100	W.I.	27.9	40.5	37.7	51.3	32.9
War (diseased)	30	D.I.	47.2	25.0	34.5	—	—
"	30	W.I.	0.7	11.0	7.0	—	—
Wolfgang von Goethe	50	D.I.	16.3	9.3	5.6	—	—
"	50	W.I.	34.2	55.0	48.1	—	—

methods of calculating delay in flowering were in general agreement. The 3 hr. steep in 0.1 % mercuric chloride delayed flowering in three or four trials out of fifteen. In this connexion it is of interest that delayed flowering was associated with increase in

TABLE 6. *Effect of treatments on date of flowering and emergence of shoots*

(Figures are numbers of experiments in which a significant delay occurred)

Treatment	No. of trials	Flowering		Emergence of shoots
		Average days method	50 % average method	
1 hr. 0.1 % HgCl_2	2	0	0	0
3 hr. 0.1 % HgCl_2	15	3	4	2
3 hr. 0.1 % HgCl_2 + meths.	3	1	1	0
3 hr. 0.1 % HgCl_2 + Agral	3	2	2	2
3 hr. 0.1 % HgCl_2 + HCl	1	1	0	0
6 hr. 0.1 % HgCl_2	1	1	1	0
16 hr. 0.1 % HgCl_2	2	1	1	0
3 hr. 0.25 % HgCl_2	2	0	1	0
Aretan, warm	10	3	3	2
Aretan, cold	4	0	0	0
Calomel	7	3	2	0
Ceresan	1	1	1	1
Folosan	3	0	0	0

weight of bulbs harvested when narcissus bulbs were treated with mercuric chloride (Hawker, 1935). It is probable that the delay in flowering permits a greater accumulation of carbohydrates and thus the production of heavier storage organs. The number of trials

with a preliminary dip in methylated spirits was small, but together with small-scale experiments these did not indicate a higher proportion of retardation with this treatment. Increase in the time of steeping tended to increase delay in flowering. The addition of Agral to the solution of mercuric chloride definitely delayed flowering, not only when compared with untreated controls but also when compared with mercuric chloride alone, in two out of three large-scale trials and in a number of small ones. It has already been pointed out that corms of var. Ave Maria which were not harmed by Agral are much harder than those of var. War and Lilac Wonder, in which injury and retardation were shown. Steeping in cold Aretan did not cause any significant delay, but warm Aretan caused delay rather more frequently than did mercuric chloride. Calomel also produced a higher proportion of delayed flowering and a greater delay than did mercuric chloride. Ceresan gave considerable delay both in one large-scale experiment and in a number of small ones. This, together with the poor quality of foliage and flowers and relatively low weight of corms, shows this fungicide to be dangerous. Folosan actually accelerated emergence of shoots in two out of three trials, but no treatment accelerated flowering.

Thus, for maximum disease control with minimum delay in flowering, a 3 hr. steep in a 0.1 % solution of mercuric chloride is satisfactory. The possibility of reducing the time to 1 hr. should be further explored.

Thanks are due to Mr J. Marshall, who carried out the greater part of the calculations involved in the analysis of variance, and to Mr T. W. Burrows, who set up preliminary experiments in 1939.

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Diseases of the gladiolus

II. Experiments on dry rot disease caused by
Sclerotinia gladioli Drayt.

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(With Plate 8)

Inoculation experiments show that (1) young corms are more susceptible than old and become less susceptible as they develop, (2) infection of unwounded corms readily takes place, (3) inoculation of the top of an old corm is more effective than that of the side or base.

The percentage diseased new corms produced from infected parent corms varies from 0 to 100 according to soil conditions. Disease is favoured by wet soil.

Removal of 'husks' (leaf bases) increases the susceptibility of corms planted in contaminated soil, but the presence of husks is not an effective control. Dehusking did not increase the number of diseased young corms in an infected stock planted in new soil.

No variety was strikingly more resistant than the rest.

Good control was obtained when corms of a diseased stock were treated with mercuric chloride, mercurous chloride (calomel) or the proprietary mercury fungicide Aretan. Calomel gave the most consistent results. Various other treatments were tested in small-scale trials with some success. Red copper oxide, formalin and the proprietary mercury compound, Ceresan, were harmful.

Sterilization of contaminated soil with formalin, mercuric chloride, Aretan or Uspulun gave good but not complete control. Formalin was the least effective.

Dry rot of gladiolus corms, caused by *Sclerotinia gladioli* Drayt., has received little attention in this country, probably owing to the relative unimportance of the gladiolus as an economic crop until recent years. The popularity of the flower increased until, by 1939, it was extensively cultivated by both commercial growers and amateurs. Large importations of corms from Holland and America, where corm diseases have been serious for some time, led to an increase in the incidence of dry rot and other diseases in Great Britain. Moore (1939) considers that a disease described by Berkeley (1867) in Kent was dry rot, but it was not until 1926 that the disease was definitely identified in this country in material from the Isles of Scilly (Pethybridge, 1929). The symptoms both in the field and on the corms have been fully described by Drayton (1926*b*), Massey (1928) and Moore (1939).

GENERAL EXPERIMENTAL METHODS

The general methods were similar to those already described (Hawker, 1944). The leaf bases ('husks') were removed from the corms when lifted, as in the experiments with hard rot. The average number of days from planting to emergence of the shoot above soil level, and the average date of flowering were calculated as in the previous paper. The amount of disease was again assessed by an arbitrary disease

index and a weight index. Special methods of inoculation and fungicidal treatment will be described in the text.

INOCULATION EXPERIMENTS

Planting corms without disease lesions in soil naturally or artificially contaminated with *Sclerotinia* invariably led to a high proportion of diseased new corms and usually to the failure of a proportion of the parent corms to produce new ones. Losses were greatest in wet soil.

Old corms at planting time and young corms during the growing season were inoculated in 1938 and 1939. The part to be inoculated was exposed and washed with sterile water or alcohol. In contact inoculations small pieces of an agar culture of *S. gladioli* were pressed against the exposed surface and covered with sterile damp cotton-wool. Small pieces of sterile agar gel were similarly applied to control plants. In wound inoculations small pieces of culture were applied to the cut surface of a corm or were inserted under a flap of tissue of a shoot or root and protected by damp cotton-wool. Controls were similarly treated except that sterile agar replaced the pieces of agar culture. Some corms were left untreated as an additional control to check the amount of disease, if any, in the stocks used. There was either some disease present in the parent corms or, more probably, the fungus spread through the

* Experiments in 1938 were by R.J.B., those in 1939 by T.W.B. Those later, including the bulk of those on fungicidal control, were by L.E.H.

soil from inoculated to control plants in a few instances, but the majority of the controls remained free from disease.

Table 1 shows that (1) young corms are more susceptible than old ones and become less susceptible as the growing season advances, (2) unwounded corms are attacked almost as freely as wounded ones, (3) inoculation of old corms at the top is more effective than inoculation at the side or base (probably due to killing of buds or young contractile roots by the fungus). Observations made during the growing

soil temperatures are normally higher than in England (Drayton, 1926*b*) (it should be noted, however, that the 1939 experiment was planted earlier than that in 1938); (4) no evidence was obtained of internal transmission of the disease from old to new corms. The lesions in the former remained shallow. The distribution of the sclerotia of the fungus and of lesions on the new corms suggested spread through the soil from old to new corms, thus agreeing with Drayton (1926*b*) and Massey (1928). The greater effect of inoculating the top rather than the side or

TABLE 1. *Inoculation experiments*

Variety	Treatment	Date of treatment	No. treated	Total new corms	No. diseased	No. severely diseased
Salmon	Old corm wound-inoculated at planting	5. v. 38	20	10	2	—*
Beauty	Old corm wounded but not inoculated	"	10	8	6	—
	Young corm wound-inoculated during growing season	27. vii. 38	10	9	9	—
	Young corm wounded but not inoculated	"	5	5	0	—
	Young corm contact-inoculated during growing season	"	10	15	13	—
	Sterile agar gel in contact with young corm	"	5	10	0	—
Lilac	None	"	20	30	10	0
Wonder	Old corm wound-inoculated at base, at planting	30. iii. 39	40	52	35	2
	Old corm wounded at base but not inoculated	"	20	29	9	1
	Old corm wound-inoculated at top, at planting	"	40	44	18	18
	Old corm wounded at top but not inoculated	"	20	33	0	0
	Old corm wound-inoculated at side, at planting	"	40	53	22	8
	Old corm wounded at side but not inoculated	"	20	30	0	1
	Young corm wound-inoculated at base	27. v. 39	5	4	2	2
	Young corm wounded at base but not inoculated	"	5	8	1	0
	Young corm contact-inoculated at base	"	5	7	5	2
	Sterile agar gel in contact base of young corm	"	5	8	1	0
	Shoot wound-inoculated 1 in. above new corm	"	5	9	4	3
	Shoot wounded but not inoculated above new corm	"	5	10	2	0
	Shoot contact-inoculated above new corm	"	5	8	1	7
	Sterile agar gel in contact shoot above new corm	"	5	10	0	0
	Young corm wound-inoculated at base	10. vii. 39	5	10	4	0
	Young corm wounded at base but not inoculated	"	5	10	0	0
	Young corm contact-inoculated at base	"	5	10	2	0
	Sterile agar gel in contact base of young corm	"	5	9	0	0
	Contractile roots wound-inoculated	"	5	10	6	0
	Contractile roots wounded but not inoculated	"	5	10	0	0
	Contractile roots contact-inoculated	"	5	10	0	0
	Sterile agar gel in contact contractile roots	"	5	11	0	0
	Young corm wound-inoculated at base	7. viii. 39	10	20	7	0
	Young corm wounded at base but not inoculated	"	10	21	2	0
	Young corm contact-inoculated at base	"	10	24	9	0
	Sterile agar gel in contact base of young corm	"	10	17	2	0

* Data not available.

season show that (1) death of the shoot occurs much more rapidly when young corms are inoculated than when old ones are inoculated at planting; (2) death appears to be due to water shortage produced by the decay of the bases of the contractile roots which are the main absorptive organs after the new corm is produced; (3) death is accelerated by hot weather; thus in 1938 death did not occur until fairly late in the growing season, but in 1939 it occurred relatively early, following a warm May, and thus more closely resembled the effects of the disease in Canada where

base of the old corm supports this view, as does the fact reported below that the proportion of diseased new corms from diseased parents is greater in wet soil, since soil conditions would be unlikely to influence internal transmission.

Incidence of infection of new corms from infected parents

Massey (1928) states that 'about 30% of offspring from diseased corms planted in clean soil are infected'. Observations during the present investiga-

tion show that this percentage varies from 0 to 100% according to soil conditions, being greatest in wet soil. Provided the soil is damp, warm weather also increases the percentage infection of new corms and the intensity of infection of individual corms. Death of the shoots, however, is delayed in wet soil, so that losses may only be apparent late in the season and yet be more severe than those in dry soil where infected individuals die early.

Effect of removal of husks on liability to infection

Drayton (1926*b*) states that diseased corms can usually be detected when lifted and that discoloured patches on the corm scales 'indicate presence of fungus... and in almost every case there will be a corresponding lesion below', i.e. on the corm surface. Massey (1928) found it necessary to remove husks before the diseased state could be determined and stated that 'the husk lesions may or may not be immediately over lesions on the corms'. The writers have found no difficulty in recognizing badly diseased corms since the husks show a tendency to split, revealing the corm surface below, and sclerotia can usually be seen on them. With slightly diseased corms it is not possible to determine the extent or nature of the disease unless the husks are removed. Moreover, surface sterilization of the corms would obviously be unsatisfactory if the husks were left intact since the fungicide might never come in contact with lesions on the corm surface. Nevertheless, it was possible that the husks might afford some protection from invasion from the soil, or might prevent or hinder the migration of the fungus through the soil from the old corm to the developing shoot, or might actually afford an easy channel of migration from old to new corms.

In a single experiment in 1938 with var. Salmon Beauty, corms with the husks removed and others with husks intact were planted directly over a sand and cornmeal culture of the fungus and at distances of 6, 12 and 18 in. from this source of infection. Table 2 gives the percentage emergence of shoots and percentage new corms diseased.

TABLE 2. *Effect of removal of husks on infection from the soil*

Distance from source of infection (in.)	No. planted	Emergence of shoots (%)		New corms diseased (%)	
		With husks	Without husks	With husks	Without husks
0	45	93.3	89.0	76.8	86.1
6	25	100	100	62.5	88.1
12	25	100	100	46.3	64.7
18	25	100	100	27.3	37.8

Thus dehusking is harmful when corms are to be planted in infected soil, which is a practice to be avoided. The incidence of infection in the progeny

of the corms with husks intact was considerable, so that the husks did not provide adequate protection against invasion of the soil.

In an experiment in 1939 with corms of var. War, more or less spotted with dry-rot lesions, skinned and unskinned batches were planted alternately. All the new corms were free from dry-rot lesions so that removal of the husks had not favoured spread of the fungus through the soil to the young corms.

Rate of spread of fungus in soil

The rate of spread of the fungus in the soil was examined in 1938, 1939, 1940 and 1941. The source of infection was naturally diseased corms or a sand and cornmeal culture. Corms free from lesions were planted at various distances from the source of infection, and other clean corms were planted at a greater distance to check the amount of disease naturally present in the stock. In the dry season of 1940 spread was not more than 9 in., but in 1939 and 1941 it was at least 15 in. but less than 18 in., while in 1938, which was a comparatively wet year, the fungus travelled at least 18 in. through the soil (Table 2). Thus, spread of the fungus is rapid, although it is likely to be influenced by soil texture, moisture and temperature, etc.

Relative susceptibility of different varieties

Drayton (1926*b*) concluded that, while all varieties are susceptible, the *Primulinus* hybrids are more so than the large-flowered *Gandavensis* type, and suggested that this might be due to the thicker corm scales of the latter. Experiments with a number of varieties wound-inoculated at planting time in 1938 or planted in contaminated soil in 1939 and 1940 suggest that this difference is largely illusory. The large-flowered varieties showed a higher proportion of old corms failing during the growing season (failure being usually due to *Botrytis* attack following injury by *Sclerotinia*). *Primulinus* varieties showed a higher proportion of diseased new corms. Thus a stock of the latter subjected to *Sclerotinia* attack would tend to multiply more rapidly than would the large-flowered types but would show a greater proportion of diseased new corms, since a larger number of seriously diseased parent corms would survive to produce infected offspring. Differences in response were also shown by number of flowers, date of flowering, total weight of new corms and weight of healthy new corms. No variety was immune or even strikingly less susceptible than the rest.

Fungicidal treatment of corms before planting

The surface sterilization of infected corms by dipping them in fungicidal solutions was attempted, with varying but usually unsatisfactory results, by van Poeteren, quoted from Moore (1939) in Holland,

TABLE 3. *Fungicidal treatment of corms before planting*

Variety	No. of corms per treatment	Index	Treatments							
			None	HgCl ₂	Calomel	Aretan warm	Aretan cold	Folosan	HgCl ₂ + Ceresan	Miscellaneous
Lilac Wonder	3 × 20	D.I.	30.0	0.2	—	0.7	0.2	20.0	—	—
	3 × 20	W.I.	59.2	62.5	—	67.1	62.8	56.7	—	—
	6 × 20	D.I.	3.4	2.0	0.6	—	—	—	0.4	—
	6 × 20	W.I.	81.9	75.9	80.2	—	—	—	80.1	—
	3 × 20	D.I.	2.2	0.2	0.0	0.0	—	—	—	—
	3 × 20	W.I.	61.5	64.3	68.6	52.3	—	—	—	—
Van Tienhoven	4 × 20	D.I.	4.1	0.5	1.3	1.9	—	—	—	—
	4 × 20	W.I.	52.5	70.0	78.9	66.9	—	—	—	—
	3 × 20	D.I.	6.5	0.5	1.2	0.7	—	—	—	—
	3 × 20	W.I.	39.1	40.3	44.0	50.6	—	—	—	—
	4 × 20	D.I.	9.4	0.0	—	0.0	5.0	0.6	—	3.0 (Ceresan)
	4 × 20	W.I.	40.0	40.8	—	32.8	44.8	50.0	—	26.9 (Ceresan)
Ave Maria	3 × 20	D.I.	8.3	3.0	2.0	—	—	—	0.9	—
	3 × 20	W.I.	37.4	41.9	48.5	—	—	—	44.6	—
	20	D.I.	10.5	1.5	5.0	—	—	—	—	—
	20	W.I.	29.5	33.0	44.5	—	—	—	—	—
	25	D.I.	10.4	0.4	0.8	3.2	—	—	—	—
	25	W.I.	31.3	32.5	40.8	35.4	—	—	—	3.3 (3 hr. 0.25 % HgCl ₂) 67.8 (3 hr. 0.25 % HgCl ₂)
Lilac Wonder	20	D.I.	11.4	1.1	—	—	—	—	—	—
	20	W.I.	77.1	83.6	—	—	—	—	—	—
	25	D.I.	21.2	12.0	7.2	1.2	—	—	—	—
	25	W.I.	43.5	51.3	47.9	42.8	—	—	—	—
	20	D.I.	28.5	23.3	0.0	—	—	—	—	41.1 (3 hr. 0.5 % formalin) 30.0 (3 hr. 0.5 % formalin)
	20	W.I.	51.0	47.0	93.0	—	—	—	—	—
Mrs Maclaren	40	D.I.	10.7	4.3	—	—	—	6.8	—	—
	40	W.I.	24.0	35.0	—	—	—	24.3	—	—
	40	D.I.	16.0	11.5	1.2	—	—	—	14.3	—
	40	W.I.	57.9	42.8	57.7	—	—	—	43.3	—
	40	D.I.	7.6	1.3	—	—	—	—	—	3.8 (HgCl ₂ + HCl) 54.5 (HgCl ₂ + HCl)
	40	W.I.	47.8	58.9	—	—	—	—	—	—
Wolfgang von Goethe	20	D.I.	15.5	5.0	—	0.5	0.0	4.0	—	—
	20	W.I.	59.0	64.5	—	77.0	67.5	62.8	—	—
	20	D.I.	6.8	0.0	—	—	—	—	—	0.5, 0.0 (Uspulun, warm and 63.5, 57.0) cold respectively
	20	W.I.	58.4	53.2	—	—	—	—	—	—
	15	D.I.	13.3	3.6	2.3	—	—	—	—	—
	15	W.I.	29.3	40.7	40.0	—	—	—	—	—
Salmon Beauty	40	D.I.	10.7	4.3	—	—	—	—	—	—
	40	W.I.	24.0	35.0	—	—	—	—	—	—
	40	D.I.	16.0	11.5	1.2	—	—	—	—	—
	40	W.I.	57.9	42.8	57.7	—	—	—	—	—
	40	D.I.	7.6	1.3	—	—	—	—	—	—
	40	W.I.	47.8	58.9	—	—	—	—	—	—
War	20	D.I.	15.5	5.0	—	0.5	0.0	4.0	—	—
	20	W.I.	59.0	64.5	—	77.0	67.5	62.8	—	—
	20	D.I.	6.8	0.0	—	—	—	—	—	—
	20	W.I.	58.4	53.2	—	—	—	—	—	—
	15	D.I.	13.3	3.6	2.3	—	—	—	—	—
	15	W.I.	29.3	40.7	40.0	—	—	—	—	—
America	40	D.I.	10.7	4.3	—	—	—	—	—	—
	40	W.I.	24.0	35.0	—	—	—	—	—	—
	40	D.I.	16.0	11.5	1.2	—	—	—	—	—
	40	W.I.	57.9	42.8	57.7	—	—	—	—	—
	40	D.I.	7.6	1.3	—	—	—	—	—	—
	40	W.I.	47.8	58.9	—	—	—	—	—	—
Prince of Wales	20	D.I.	15.5	5.0	—	0.5	0.0	4.0	—	—
	20	W.I.	59.0	64.5	—	77.0	67.5	62.8	—	—
	20	D.I.	6.8	0.0	—	—	—	—	—	—
	20	W.I.	58.4	53.2	—	—	—	—	—	—
	15	D.I.	13.3	3.6	2.3	—	—	—	—	—
	15	W.I.	29.3	40.7	40.0	—	—	—	—	—

by Massey (1928) in U.S.A. and by Drayton (1926*a*, 1926*b*) in Canada. Drayton suggested that dipping for 15 min. in a 10% solution of Uspulun or Semesan at 50° C. before planting might be worth while with small quantities of valuable varieties, while McCulloch (McCulloch & Weigel 1941) recommended disinfection with mercuric chloride, mercurous chloride (calomel) or certain organic mercury compounds as a control measure against corm diseases in general.

The fact that spread from the old corm to the new takes place externally suggests that control would be achieved not only by complete destruction of the fungus in the corm lesions, but also by coating the old corm with sufficient fungicide to prevent the fungus from growing out into the soil. A combination of surface sterilization of the corm with the addition of a fungicide to the surrounding soil would probably be more effective than corm treatment

of small-scale ones, in the seasons 1940, 1941, 1942 and 1943 tested the following treatments: (1) 3 hr. in a 0.1% solution of mercuric chloride (HgCl_2); (2) 15 min., or later 1 hr., in a 0.5% solution of Aretan (Aretan, warm); (3) 3 hr. in a 0.5% solution of Aretan (Aretan, cold); (4) 5 min. in a 1.0% suspension of mercurous chloride, the suspension being stirred all the time (calomel); (5) dipped in water and rolled in Folosan (Folosan); (6) treated as (1) but planted in a handful of a 1:10 mixture of Ceresan and dry soil (HgCl_2 + Ceresan). Dusting the corms with Ceresan or steeping them for 3 hr. in 0.1% mercuric chloride with the addition of 1.0% concentrated hydrochloric acid or in 0.5% formalin or 0.5% Uspulun or for 15 min. in the latter at 50° C. were tried in a few small-scale experiments. The corms were all more or less spotted with dry-rot lesions, and the batches undergoing various treat-

TABLE 4. *Comparison of pairs of treatments*

Treatments compared	No. of trials	Disease index		Weight index	
		2nd treatment significance better	No difference	2nd treatment significance better	No difference
None v. HgCl_2	7	7	0	1	6
None v. Aretan, cold	2	1	1	0	2
None v. Aretan, warm	5	5	0	2	1
None v. Calomel	5	5	0	2	3
None v. HgCl_2 + Ceresan	2	2	0	0	2
None v. Folosan	2	1	1	0	2
Calomel v. HgCl_2	5	0	4	0	3
Aretan, cold v. HgCl_2	2	0	2	0	2
Aretan, warm v. HgCl_2	5	0	5	2	1
HgCl_2 + Ceresan v. HgCl_2	2	0	2	0	2
Folosan v. HgCl_2	2	1	1	0	1
Cold Aretan v. warm Aretan	2	1	1	0	1

alone but would involve extra labour and expense. Accordingly, a wide variety of corm treatments sometimes combined with soil treatment were tested in 1938 and 1939. These trials were not usually replicated, and the disease index and weight index could not be calculated since the causes of failure were not always determined with certainty. Nevertheless, these experiments indicated that corm treatment with mercuric chloride, the proprietary mercury compounds Aretan or Uspulun or the non-mercury proprietary compounds Folosan or Brassisan, reduced the amount of disease in the new corms. Treatment with formalin, red copper oxide or the proprietary mercury compounds Ceresan or Improved Ceresan were shown to be harmful. The combination of corm treatment with dusting the trenches with Folosan or Brassisan or watering them with fungicidal solutions did not give increased control over corm treatment alone.

Replicated experiments, together with a number

ments were matched as nearly as possible. It is clear from Table 3 that treatment with mercuric chloride, warm Aretan or calomel was beneficial. Calomel gave the most consistent results, which was to be expected since a thick deposit of the fungicide was left on the corms, thus preventing spread of the fungus to the new corms. It is significant that, where calomel gave a relatively high disease index, this was due to failure of old corms rather than to a high proportion of disease among the new ones. Table 4 shows that the beneficial effects of these treatments were significant when measured by disease index, but that the improvement in the weight index was much less marked than with hard rot (Hawker, 1944). Results with Folosan and cold Aretan were not consistent, but suggest that while these treatments give some control they are sometimes inferior to treatments (1), (2) and (3). The use of Ceresan in the soil surrounding the corms did not give additional control.

In the previous paper (Hawker, 1944) it was shown that corm treatment with mercuric chloride, Aretan or calomel sometimes caused a significant delay in flowering which was often associated with an increase in the weight of new corms produced. These treatments had no adverse effect on quality of foliage or flowers. Ceresan was harmful, and in the preliminary experiments described above Improved Ceresan and red copper oxide were even more toxic. Formalin invariably caused increase in the number of diseased corms but was not otherwise harmful.

Fungicidal treatment of contaminated soil

Since the rate of spread of *Sclerotinia* in the soil is rapid, soil soon becomes heavily contaminated from the presence of even a low proportion of infected corms in a stock. Thus it is not always practicable to plant in soil absolutely free from the disease organism. Attempts at soil sterilization were not

or partially brown, while those in the treated pots were green and healthy. Table 5 shows the length and condition of the shoots at this time (when the surviving plants were transplanted into a plot which had not grown gladioli before) and also shows that none of the plants from the control pots flowered and that these either failed to produce new corms or produced a few heavily diseased ones while the treated lots produced a good crop of healthy or slightly diseased corms.

The following plots were also treated:

(1) A 6 ft. sq. plot from which a severely diseased crop had been lifted in 1939 was divided into four 3 ft. sq. plots by wooden partitions sunk about 9 in. into the ground. Two sections were left untreated and the other two were given 6 g. Uspulun/sq. ft. (applied as a dilute solution) and 20 c.c. commercial formalin/sq. ft. (applied as a dilute solution) respectively in February 1940. The formalin-treated section

TABLE 5. *Pot experiment. Fungicidal treatment of artificially contaminated soil*

	Treatment			
	None	HgCl ₂	Uspulun	Formalin
Average length leaves in cm. 16. v. 41	15.5	51.7	45.4	44.8
No. shoots completely brown 16. v. 41	8	0	0	0
No. plants rotting at base of shoot 16. v. 41	9	0	0	0
Total flowers	0	8	10	6
Disease index	51.1	0.6	1.2	1.8

TABLE 6. *Soil treatment of two plots*

Plot	1939	1940	1941	1942	1943
1 A	Diseased crop	None	None	None	None
B	"	Uspulun	Uspulun	Uspulun	"
C	"	Formalin	Formalin	Formalin	"
D	"	None	None	None	"
2 A	"	Inoculated <i>Sclerotinia</i>	None	None	Half-treated Aretan
B	"	"	Aretan	Aretan	"
C	"	"	Formalin	Formalin	Half-treated formalin
D	"	"	None	None	"

successful under Canadian conditions (Drayton, 1926*a*, 1926*b*).

Autoclaved soil was contaminated with a sand and cornmeal culture of *Sclerotinia* and left for 3 weeks in a warm greenhouse to allow the fungus to spread through the soil. The soil was turned over and watered several times during this period. Twelve 10 in. pots were then filled with the contaminated soil and treated as follows: (1) three pots left untreated, (2) three pots each given 1 l. of a 0.1% solution of mercuric chloride, (3) three pots each given 1 l. of a 0.5% solution of Uspulun, (4) three pots each given 1 l. of a 5.0% solution of formalin. Three weeks later six lesion-free corms of var. Van Tienhoven were planted in each pot. Pl. 8, figs. 1-4, shows these pots 4 months later, and it is clear that all three treatments were beneficial. The difference was actually greater than the photographs show, since the shoots in the control pots were either completely

was covered with sacks for 1 week. The control sections were watered with a volume of water equal to the volume of fungicide applied to the treated ones. Fifty lesion-free corms of var. Lilac Wonder were planted in each section in March 1940. The plot was similarly treated and planted in 1941 and 1942 but was left untreated in 1943 (Table 6). Table 7 shows that both treatments brought about a considerable reduction in the amount of disease as shown by both disease index and weight index. Formalin was less effective than Uspulun. In 1941, which was a relatively wet year, disease both in control and treated lots was more severe than in the dry year of 1940. The effect of the treatments persisted in 1943 when the plot received no treatment. Pl. 8, figs. 5-7, show that the treated lots were flowering when the buds in the control sections were unopened. The total number of flowers was greater each year in the treated lots.

(2) A similar sized plot from which a lightly diseased crop was lifted in 1939 was spread with a sand and cornmeal culture of *Sclerotinia* (which was lightly forked in) early in 1940 and planted with lesion-free corms which became severely diseased. The plot was divided up similarly to plot 1 in 1941. One section was treated with formalin as in plot 1 and another was given 6 g. of Aretan/sq. ft. These treatments were repeated in 1942, but in 1943 each section was further divided into two parts and treated according to the scheme in Table 6. Both treatments gave a considerable reduction in the amount of disease (Table 7). Formalin again was inferior to the mercury compound. The 1943 results show that a single treatment is beneficial but suggest that the

than in the lighter soil of plots 1 and 2, the treatments again gave a considerable improvement. This was not sufficient to be satisfactory, however, particularly in the wet year of 1941. Flowering was again retarded and reduced by disease in the control lots.

(4) Sections of a plot similar to plot 1 were treated 10 oz./sq. yd. of the non-mercury fungicides Folosan and Brassisan. These gave no control, and a number of small-scale experiments in which these fungicides were applied to the soil in various ways also gave negative results.

Corm treatment with calomel before planting in contaminated soil reduced the number failing to grow (with a consequent favourable effect on the weight index), but the amount of disease among the

TABLE 7. Incidence of disease in the two plots of Table 6

Plot	1940		1941		1942		1943	
	D.I.	W.I.	D.I.	W.I.	D.I.	W.I.	D.I.	W.I.
1 A	11.8	16.4	46.3	0.12	23.9	12.4	23.2	11.2
B	0.2	37.2	8.3	53.3	1.6	45.4	4.7	84.1
C	0.6	43.1	21.5	39.3	6.5	32.2	16.0	25.8
D	10.4	17.5	50.2	2.5	29.0	10.8	26.5	10.4
							Untreated half	
							Treated half	
							D.I.	W.I.
2 A	—	—	*46.5	7.6	14.9	28.5	31.1	8.1
B	—	—	*11.9	29.8	0.0	59.6	5.9	39.0
C	—	—	*13.0	14.2	3.2	55.2	14.0	41.2
D	—	—	*54.0	3.7	10.2	34.1	22.1	18.2

* Planted Van Tienhoven, rest planted Lilac Wonder.

D.I. = disease index, W.I. = weight index.

TABLE 8. Effect of fungicidal treatment of soil in an artificially contaminated plot

Variety	No. corms per treatment	Year	Index	Treatment			
				None	Formalin	Uspulun	Aretan
Lilac Wonder	30	1941	D.I.	93.0	46.4	38.1	—
"	30	1941	W.I.	0.1	16.8	43.0	—
America	50	1942	D.I.	31.6	14.3	—	16.4
"	50	1942	W.I.	3.5	11.4	—	17.0

disease would soon become re-established unless treatment was repeated at yearly intervals. Pl. 8, fig. 8, shows an earlier flowering in the treated lots as in plot 1.

(3) A 9 × 3 ft. plot of heavier soil than that of plots 1 and 2 was spread with a sand and cornmeal culture of *Sclerotinia* (which was forked in) in January 1941. The plot was then divided into strips, one of which was left untreated, one was Uspulun-treated and the third formalin-treated at the same rate as plot 1. In 1942 the top soil was removed, thoroughly mixed and replaced. Strips, at right angles to those of 1941, were treated with formalin and Aretan (as in plot 2) and left untreated respectively. Table 8 shows that, although the amount of disease was generally much greater in this wet plot

new corms was high. This was to be expected in view of the greater susceptibility of the young corms.

Thus, although corms should not be planted in contaminated soil yet, if this be unavoidable, a good measure of control may be obtained in normal years in a light soil by the use of formalin or of a mercury fungicide. Formalin gives less control but is preferable owing to the danger of harmful effects from frequent application of mercury compounds to the soil.

The soil treatments were also applied to a plot where gladioli had not previously been grown so that the effects on quality and date of flowering and on weight of new corms would not be complicated by the presence of disease. No treatment was harmful. Flowering was not accelerated by any

treatment, so that the difference in time of opening seen in Pl. 8, figs. 5-8, was due to the retarding effect of disease in the control lots.

The writers are indebted to Mr J. Marshall for carrying out the bulk of the routine calculations in connexion with the analysis of variance.

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EXPLANATION OF PLATE 8

Fig. 1. Soil contaminated with *Sclerotinia gladioli*. Each pot planted with six lesion-free corms of var. Van Tienhoven. Note stunted plants.

Figs. 2-4. Soil as in fig. 1 but treated with 1 l. per pot of 0.1 % mercuric chloride, 0.5 % Uspulun and 5.0 % commercial formalin respectively. Note well-developed foliage (see Table 5).

Fig. 5. Plot 1 of Tables 6 and 7. Arrowheads show wooden partitions dividing plot. Sections front left (C) soil treated with formalin, rear left (A) and front right (D) untreated, rear right (B) treated with Uspulun. Note retardation of flowering in sections A and D.

Fig. 6. Plot 1 seen from corner thus: front section (A) and rear section (D) untreated, right-hand section (C) treated with formalin, left-hand section (B) treated with Uspulun.

Fig. 7. Sections (D) untreated (in foreground) and (B) treated with Uspulun (in rear) of plot 1.

Fig. 8. Plot 2 of Tables 6 and 7. Sections front left (C) treated with formalin, rear right (B) treated with Aretan, rear left (A) not visible and front right (D) untreated. Note retarded flowering in untreated sections as in plot 1.

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The short wet method of seed disinfection

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(With Plate 9)

An account is given of the use of the short wet method of seed disinfection for the prevention of *Helminthosporium* disease of oats. Equally good results were obtained by its use as those given by the dusting method. A soluble organo-mercury preparation (Ceresan, U. 564) was employed as the disinfectant. Suitable machinery is necessary for carrying out the short wet method of treatment, and two of the machines manufactured for this purpose are described. They are the 'Primus', a hand-operated machine for dealing with small quantities ($\frac{3}{4}$ cwt. lots) of seed, and the 'Kontramix', a power-driven machine designed for carrying out continuous seed treatment at rates varying from 5 to 16 cwt./hr.

INTRODUCTION

Whereas the dusting method of seed disinfection, when properly applied and using a reliable organo-mercury preparation, is extremely effective, it is felt that there may be a real need for an alternative

method whereby the use of a dust is avoided. This is particularly so where large crop acreages are sown by hand and where the presence of dust, whether poisonous or not, mixed with the seed may prove unpleasant during sowing operations. Such unpleasantness may be eliminated by using a form of



wet treatment. Until comparatively recently the technique of wet treatment involved either the steeping of the seed or sprinkling it with a solution of the disinfectant, but in either case it was necessary to dry the seed thoroughly after treatment. The possibility of seed injury by the disinfectants commonly used and the extra labour involved in the drying operation were distinct drawbacks and gave the dusting method a decided advantage. The introduction of the short wet method, however, following upon the work of Gassner (1927), and using a solution of an organo-mercury compound as the disinfectant did away with the necessity for a special drying operation and minimized the risk of seed injury so that the advantages hitherto enjoyed by the dusting method were no longer so apparent. The principle of the short wet method is the same as that for steeping or sprinkling except that the quantity of liquid used is so small that the seed dries out readily after resacking without suffering any deleterious effect. The thorough wetting of the seed by such a small quantity of liquid is achieved by the inclusion of an effective wetting agent with the disinfectant.

SMALL-SCALE EXPERIMENTS

In 1935 the writer's attention was drawn to 'Ceresan, U. 564', a water-soluble disinfectant containing organo-mercury (3.5 % metallic mercury) as its active constituent and regarded as suitable for use with the short wet method. Although in the form of a white powder it gives a deep blue solution in water owing to the incorporation of a tracer dye. Small-scale trials were made with this material from 1935 to 1937 employing the steeping, sprinkling and short wet methods and testing the effectiveness of the disinfectant against *Helminthosporium* disease of oats.

DETAILS OF METHODS USED

Steep. 0.2 % solution; seed steeped for 30 min. and then dried.

Sprinkle. 0.5 % solution; 1.3 gal./cwt. (120 c.c./kg.) of seed; seed sprinkled and then dried.

Short wet. 3.5 % solution, 0.45 gal./cwt. (40 c.c./kg.) of seed.

The testing technique employed was that devised by the writer (Muskett, 1938), and the results obtained were compared with those given by the dusting method using 'Ceresan, U.T. 1875 A' at the rate of 2 oz./bushel (approx. 3 g./kg.) and also formalin (1 in 320) used by the sprinkle method at the rate of 1 gal./cwt. (approx. 90 c.c./kg.) of seed (see Table 1).

RESULTS

Table 1 shows that the short wet method of treatment using 'Ceresan, U. 564' as the disinfectant is as effective as the dusting method for preventing *Helminthosporium* disease of oats. Numerous small-scale trials have been made using varying concen-

trations of the solution applied at different dosage rates, but in so far as the prevention of this disease is concerned no improvement was effected upon the concentration of 3.5 % applied at the rate of 0.45 gal./cwt. of seed.

Preliminary small-scale trials made with another proprietary material containing a soluble organo-mercury compound (Sch. 3330) and suggested as being suitable for application by the short wet method, also gave promising results.

Solutions of anhydrous sodium carbonate and glacial acetic acid used up to and including concentrations of 8.0 % proved to be quite ineffective when applied by the short wet method against *Helminthosporium* disease.

TABLE 1

		Laboratory test	First pot test (moist soil)	Second pot test (dry soil)	Field test
(expressed as % primary infection with <i>H. Avenae</i>)					
1935	Control	42.8	44.0	48.0	30.0
	Formalin sprinkle	9.2	12.0	15.0	12.0
	U.T. 1875 A (dust)	1.0	0.0	0.0	0.8
	U. 564 short wet	—	0.0	0.0	1.7
	U. 564 sprinkle	—	0.0	0.5	3.3
	U. 564 steep	—	0.0	0.0	6.9
1936	Control	43.0	21.0	42.0	5.0
	Formalin sprinkle	20.6	6.0	26.2	3.2
	U.T. 1875 A (dust)	0.0	0.0	0.0	0.0
	U. 564 short wet	0.2	0.0	0.0	0.2
	U. 564 sprinkle	5.0	0.2	0.8	0.6
	U. 564 steep	0.2	0.0	0.0	0.2
1937	Control	20.0	19.8	14.4	23.8
	Formalin sprinkle	19.0	12.8	11.6	13.0
	U.T. 1875 A (dust)	0.0	0.0	0.0	0.3
	U. 564 short wet	0.0	0.0	0.2	0.3
	U. 564 sprinkle	12.0	0.6	0.6	0.3
	U. 564 steep	0.0	0.0	0.0	0.1

Large-scale experiments

Small quantities of seed for experimental work can readily be submitted to the short wet treatment by shaking the seed and disinfectant together in a large Erlenmeyer flask, but the treatment of seed in bulk by the simple method of spreading it in a shallow heap and spraying it with the required quantity of the disinfectant, while the heap is being continually mixed, is difficult in practice. The quantity of liquid is small and it is almost impossible to ensure even mixing and the wetting of every seed with the disinfectant. Tests made with seed so treated invariably yielded results inferior to those obtained by the use of other methods. An efficient machine is necessary for carrying out the treatment on a large scale and, during the course of this work, it has been possible to submit to thorough trial two machines especially designed for carrying out the

treatment. Both machines are of German manufacture, one being the 'Primus', a small hand-operated machine manufactured by G. Drescher of Halle (Salle) and the other, the 'Kontramix', a power-driven machine manufactured by W. Lambach of Marienhyde (Rhineland).

The 'Primus' (Pl. 9, figs. 1, 2). The small hand-operated model used in this work is capable of dealing with some $\frac{3}{4}$ cwt. (38 kg.) of seed oats at a time. It is of the revolving drum type, and turning for a period of 3 min. is required to complete the mixing operation. The necessary quantity of liquid disinfectant is fed into a container mounted on one end of the hollow axle which is perforated at intervals to allow the liquid to escape into the body of the drum and become mixed with the seed (Pl. 9, fig. 2). As the drum revolves, a valve actuated by a cam on the axle, and placed at the base of the container, comes into operation, and each time it opens a quantity of the liquid leaves the container and enters the axle. The mixing is assisted by the action of a series of paddles or baffle plates fixed to the axle. Providing the perforations in the axle are covered with adhesive tape or some other suitable material, the machine can be used for the dusting treatment of seed. In the course of this work the 'Primus' has been found to be quite effective and satisfactory for carrying out the short wet treatment. Many tests have been made with samples of seed oats heavily contaminated with *H. Avenae*, and results comparable with those obtained in small-scale experiments have always been achieved.

The 'Kontramix' (Pl. 9, figs. 3-5). This machine, which is power-driven (consumption approx. 0.33 h.p.), is designed for the continuous treatment of seed by the short wet method. The treatment rate varies from about 5-16 cwt. of seed per hour according to the type of seed used and the rate desired. The grain is fed in at the top of the machine through a large hopper, the rate of its entering being regulated by the adjustment of a movable slide. It then falls into the deep grooves of a rotating cylinder by which means an even and controlled rate of feed is ensured. From the grooves of the cylinder it passes into the disinfection chamber through a copper funnel which directs its fall on to the sloping sides of a metal cone fixed towards the top and in the middle of the chamber. This results in its being dispersed towards the sides of the cylindrical chamber through which it continues to fall in the form of an annulus. The atomizing nozzle through which the disinfectant is sprayed in the form of a fine mist is fixed in an upright position at the bottom centre of the chamber so that, when the machine is in operation, the falling seed meets and passes through the cone of misty spray (Pl. 9, fig. 5). The wetting of each individual seed is thereby ensured and the mixing of the seed is achieved in the short mixing trough into which it then falls (Pl. 9, figs. 4, 5). Throughout the length

of the mixing trough a revolving screw, to which are attached eight blades and two mixing attachments, operates during the process of disinfection (Pl. 9, fig. 4). By this means the seed is thoroughly mixed and, at the same time, slowly propelled towards the openings to which are attached the sacks. As each sack is filled it is removed and set aside for sewing and labelling. The seed dries in the sack and within a few hours of treatment is ready for sowing.

The disinfectant is contained in a 110 l. tank (110 l. is sufficient for the treatment of approximately 2.5 tons of seed oats), at the bottom of which is a very fine filter through which the liquid must pass to reach the single piston pump which drives it under high pressure to the atomizing nozzle. Attached to the nozzle support is another system of fine sieves through which the disinfectant passes before reaching the nozzle. Two nozzles of differing apertures are supplied, one for use at the lower treatment rates and the other when the treatment rate is high. Within the limits of the treatment rates of which each nozzle is capable, the delivery rate of the disinfectant is regulated by changing the pressure through the operation of a ball valve attached to the nozzle support. The valve ball is depressed to varying depths by the action of a sliding weight acting along a lever arm, and by shifting the position of the weight the pressure, and consequently the quantity of liquid delivered, can be regulated with great accuracy; the excess liquid passes back into the tank. When once the rate of seed flow through the machine has been determined, the quantity of liquid required for disinfection and the appropriate position of the sliding weight on the lever arm necessary to ensure the delivery of this quantity, may be read immediately from a chart supplied.

Repeated tests have been made with the 'Kontramix' during the past 7 years and, in so far as the control of *Helminthosporium* disease of oats is concerned, the results given by its use have compared very favourably with those from any other method employed. Machines of this type are installed at the Plant Breeding Division, Stormont, and at the Institute of Agricultural Research, Hillsborough, where the disinfection of all cereal seeds by the short wet method has not become general farm routine. Experiences at both these centres show that the avoidance of the use of dust is regarded as a distinct advantage and that the short wet method is generally preferred.

GENERAL CONSIDERATIONS

The question of the storage of cereal seeds after treatment by the short wet method has not been investigated fully, but information gained indicates that the germination capacity of the seed remains unimpaired after storage for at least 3 months. It is recommended, therefore, that until further evidence becomes available, seed so treated should be sown



Fig. 1

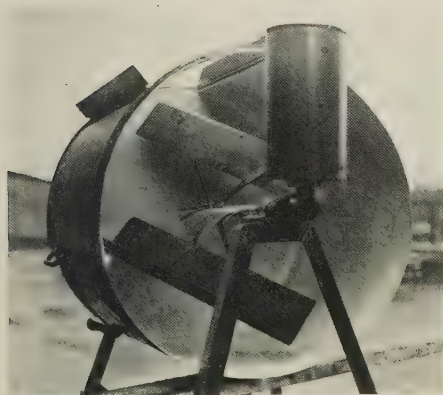


Fig. 2

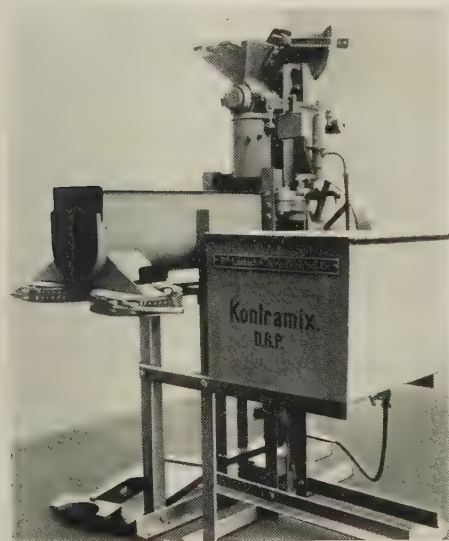


Fig. 3



Fig. 4

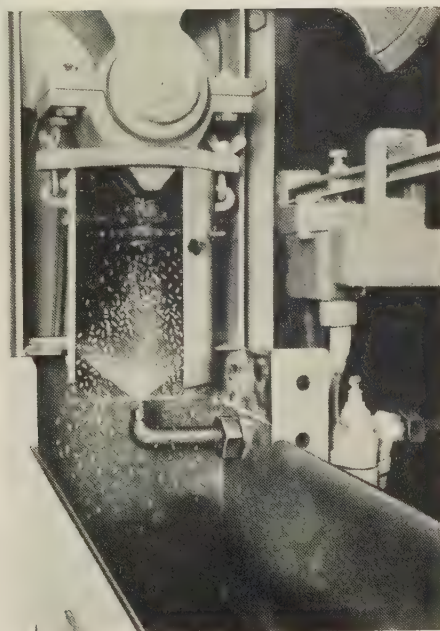


Fig. 5

within 3 months of its having been disinfected. The fact that some 4.0% of moisture (calculated upon the original weight of the seed) is added during treatment does not appear to be of significance in general practice using normal undried seed samples. Some of this extra moisture is lost by evaporation, and if the seed is stored under dry conditions with good ventilation it will tend to dry out further during storage. Heating has never been observed under any

circumstances. From any costings made so far, the cost of disinfection by this method compares favourably with that of the dry treatment.

Due acknowledgement is made to Messrs Bayer Products, Ltd., of London, and in particular to Mr C. T. N. Wakely, for assistance in obtaining the machines used in this investigation and also for the original photographs used for Pl. 9, Figs. 2-5.

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- MUSKETT, A. E. (1938). Biological technique for the evaluation of fungicides. I. The evaluation of seed disinfectants for the control of *Helminthosporium* disease of oats. *Ann. Bot., Lond.*, N.S. 2, 699.

EXPLANATION OF PLATE 9

- Fig. 1. The 'Primus' hand-operated machine. The container holding the disinfecting solution is seen to the left of the rotating drum containing the seed grain.
- Fig. 2. The drum of the 'Primus' with one end removed, showing the mixing paddles and the disinfecting solution being delivered from the perforations in the hollow axle. The container is shown in the foreground.
- Fig. 3. A general view of the 'Kontramix' power-driven machine; the large hopper for containing the grain which is fitted to the top of the machine is not shown.

- Fig. 4. One great advantage of the 'Kontramix' is the rapidity with which it can be thoroughly cleaned after use when it may be desired to change the species or variety of cereal requiring treatment. The photograph shows the mixing apparatus removed from the mixing trough and being cleaned.
- Fig. 5. Here a portion of the cylinder through which the grain falls has been removed and the grain can be seen falling through the cone of atomized spray into the mixing trough. To the right is the lever arm and movable weight by the adjustment of which the rate of flow of the disinfecting solution is regulated.

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A comparison of the toxicity of certain dyestuffs to the conidia of *Fusarium culmorum*

By W. M. DION AND K. A. LORD, *Rothamsted Experimental Station, Harpenden, Herts*

The toxicity of a number of dyestuffs to the spores of *Fusarium culmorum* and *Cercospora herpotrichoides* was determined by the slide-germination technique. No attempt was made to distinguish between fungistatic and fungicidal activity.

The toxicity of basic dyestuffs was unaffected by the acid radicle associated with the dye base.

The high toxicity to *Fusarium culmorum* of malachite green dye base was reduced weight for weight and mole for mole by substitution of ethyl, propyl or butyl groups for methyl groups.

The reduction of malachite green to malachite green *leuco* base removed toxicity.

The substitution of amino groups and alkylated amino groups in benzene nuclei of triphenyl methane increased toxicity, whereas acid groups reduced toxicity. Sulphonation and carboxylation reduced toxicity to vanishing point.

Alkylation of amino groups increased, but alkylation of benzene nuclei did not affect toxicity appreciably.

When the central carbon atom of the triphenyl methane dyestuffs was replaced by nitrogen (e.g. Bind-schedler's green) the diphenyl ammonium compounds were less toxic than the corresponding triphenyl-methane compounds.

The prevention of rotation of the aminated benzene rings by bridging, in the *o*-position to central atom, with O or N, and so obtaining a planar molecule only slightly affected toxicity.

Certain acid dyes stimulated fungal growth.

The toxicity of the basic dyestuffs seems to depend not on one specific part but on the molecule as a whole, and within certain limits the structure may be varied without pronounced changes in toxicity.

INTRODUCTION

The toxic action of dyestuffs, especially of malachite green, on bacteria and fungi has been recognized for some time, but comparatively little practical use has been made of them in plant pathology.

In the course of testing a number of substances for the control of eyespot of wheat caused by *Cercospora herpotrichoides* Fron., Glynne (unpublished work) found that malachite green and crystal violet were more toxic than some other dyes. The present work was undertaken to determine whether any correlation could be found between toxicity and chemical structure.

In this investigation the spores of *C. herpotrichoides* were initially used, but this fungus was soon abandoned on account of the difficulties in obtaining spores under standard conditions. The factors controlling spore production in culture are not fully understood, and the spores used were therefore obtained from several different isolations. Even with this additional source of error the results when duplicated did not show much variation, and are given for comparison with the results obtained with *Fusarium culmorum*.

METHOD

The toxicity of the fungicides was estimated by the standard slide-germination technique first used by Reddick & Wallace (1910) and which has since been developed by a number of workers, notably McCallan, Wellman and Wilcoxon (see McCallan *et al.* 1941; McCallan & Wilcoxon, 1939; Wellman & McCallan, 1942).

Great care was taken to eliminate all avoidable sources of variation. The glassware was cleaned with chromic acid between each test, and the fungus spores were standardized as far as was possible. The test fungus used was *F. culmorum* isolated from potato in 1943. A single spore isolate was made and a number of stock cultures was established from it and grown on potato dextrose agar at 17° C. The spores used were always from the first transfers from the stock cultures and were taken 12-15 days from the date of transfer.

The spores were obtained by gently washing the surface of the mycelium with sterile tap water, and the suspension was diluted until sample drops contained approximately twenty spores under the low-power objective. On account of the prolific production of spores by this fungus very little washing was necessary to obtain the required amount. In these tests tap water was used as a stimulant for germination, as growth was irregular in distilled water. Owing to the variation in the composition of tap water a large quantity was sterilized at one time and used throughout all the tests.

Several of the substances tested were not soluble in water, so all solutions were made up in sterile

distilled water and alcohol, the concentration being adjusted so that the medium in which the spores germinated contained 5 % alcohol in every case. The same adjustment was made for the controls.

One c.c. of the test solution was added to 1 c.c. of the spore suspension in a test-tube, and after being shaken the solutions were pipetted out on to glass slides, so that there were five drops of approximately equal size on a slide. A fresh slide was used for each solution. The slides were arranged on glass racks in inverted Petri dishes which were sealed with water to form damp chambers.

The spores were incubated at 25° C. for 24 hr. and then examined for germination. The normal growth during this period was 30-40 times the original spore length. As the critical toxic concentration was approached the spores produced germ tubes shorter than the original spore length. The initial tests of dyestuffs were carried out over the following range of concentrations, 1, 5, 10, 50, 100, 500, 1000 p.p.m. since the toxicity of the substances fell within this wide range, and usually one concentration had no toxic effect while the next was completely toxic. In order to observe the gross differences with which we were concerned it was not considered necessary to determine the L.D. 50 for each substance. A more refined technique would, however, be necessary to ascertain the smaller changes in toxicity resulting from slight variations of molecular structure. In addition, no attempt was made to differentiate between fungistatic and fungicidal action.

All the tests were repeated at least twice at different times, and where there was variation they were repeated until three or more gave the same result. In general, the agreement between tests was remarkable.

TABLE 1. *Comparison of toxicity of salts and bases of certain dyestuffs to spores of Fusarium culmorum*
Concentrations expressed in terms of the bases in p.p.m.

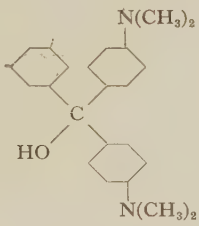
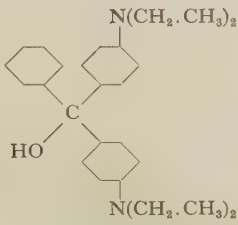
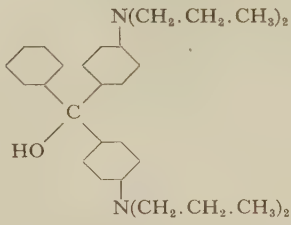
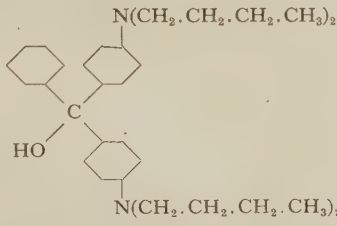
First figure: 100 % germination approximately.
Second figure: germination completely inhibited.

Methyl violet 2B				
Base	Hydrochloride	Sulphate		
1-5	1-5	1-5		
Magenta (CH ₃ group in one ring)				
Base	Hydrochloride	Sulphate		
10-50	10-50	10-50		
Malachite green				
Base	Hydrochloride	Sulphate	Oxalate	Picrate
1-10	1-10	1-10	1-10	0.5-10
Mischler's hydrol				
Base	Hydrochloride			
50-100	50-100			
Auramine				
Base	Hydrochloride			
50-500	50-500			

TABLE 2. *The effect of substituents in the amino groups of malachite green upon its toxicity to the spores of Fusarium culmorum and Cercospora herpotrichoides*

Concentrations expressed in terms of the bases.

First figure: 100 % germination } concentrations of dyestuff in p.p.m.
 Second figure: no germination }

		<i>Fusarium</i>	<i>Cercospora</i>
Malachite green Tetramethyl- <i>p</i> -diamino- triphenyl carbinol		1-10 (2.9-29)	1-10 (2.9-29)
Brilliant green Tetraethyl- <i>p</i> -diamino- triphenyl carbinol (sulphate)		1-50 (2.5-124)	1-5 (2.5-12.4)
Tetra- <i>n</i> -propyl- <i>p</i> -diamino- triphenyl carbinol (chloride)		10-100 (22-220)	1-10 (2.2-22)
Tetra- <i>n</i> -butyl- <i>p</i> -diamino- triphenyl carbinol (chloride)		10-500 (19-970)	10-50 (19-97)

(Numbers given in brackets are g.mol. in 10³ c.c. of solution.)

TABLE 3. The toxicity of a number of di- and triphenyl methane derivatives to the spores of *Fusarium culmorum* and *Cercospora herpotrichoides*

Concentrations expressed in terms of the bases.

 First figure: 100 % germination } concentration in p.p.m.
 Second figure: no germination }

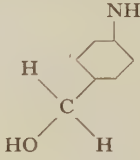
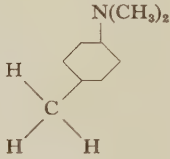
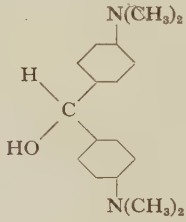
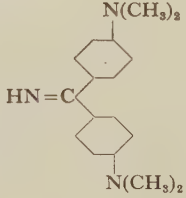
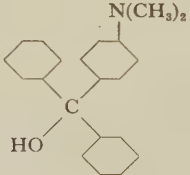
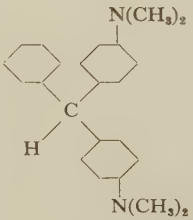
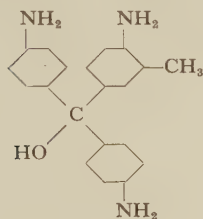
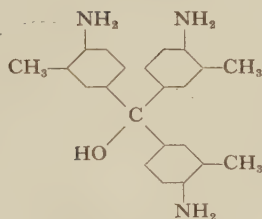
		<i>Fusarium</i>	<i>Cercospora</i>
Aniline hydrochloride		Non-toxic at 1000 p.p.m.	—
<i>p</i> -Aminobenzyl alcohol		Non-toxic at 1000 p.p.m.	—
Dimethyl- <i>p</i> -toluidine		Non-toxic at 1000 p.p.m.	—
Mischler's hydrol (highest conc. in 10 % alcohol) (base chloride)		50-100	—
Auramine (base chloride)		50-500	—
Dimethyl- <i>p</i> -amino-triphenyl carbinol (highest conc. in 10 % alcohol) (chloride)		Reduces growth	—
Leuco-malachite green (highest conc. in 10 % alcohol) (chloride)		Non-toxic at 500 p.p.m.	—

TABLE 3 (continued)

Magenta (base, chloride, sulphate)

*Fusarium*
10-50*Cercospora*
1-5

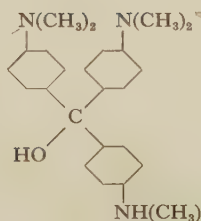
Magenta (chloride)



10-50

5-10

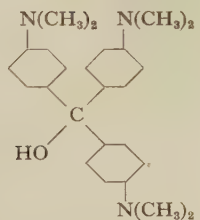
Methyl violet 2 B (base, chloride, sulphate)



1-5

0.5-1

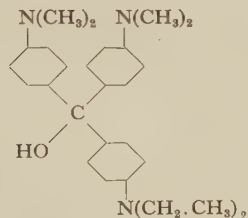
Crystal violet (chloride)



1-5

0.1-10

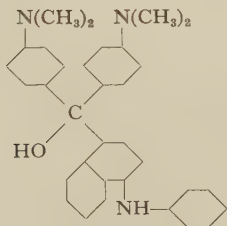
Crystal violet (chloride)



0.5-5

—

Victoria blue (chloride)



1-5

1-10

TABLE 3 (continued)

		<i>Fusarium</i> 1-5	<i>Cercospora</i> 0.5-5
Night blue (chloride)			
Aniline blue (spirit soluble) (chloride)		Shows signs of toxicity by reducing growth and giving some kill, but never 100 %	5-50
Setocyanine (chloride)		1-10	1-5
(—)* (chloride)		10-50	—
Acriflavine (chloride)		5-50	—
Aurine		100-500	—

* The toxicity of this compound, for which we have not been able to trace a name, should be compared with that of brilliant green (Table 2).

TABLE 4. Toxicity of certain basic dyestuffs to the spores of *Fusarium culmorum* and *Cercospora herpotrichoides*

(Nitrogen replacing the central carbon atoms of the di- and triphenyl methane dyestuffs.)

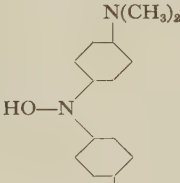
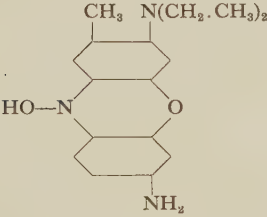
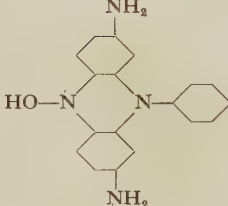
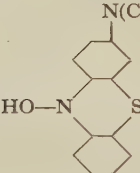
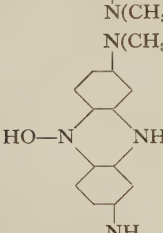
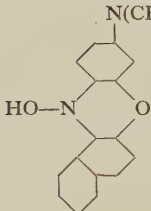
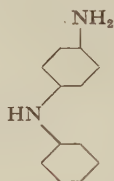
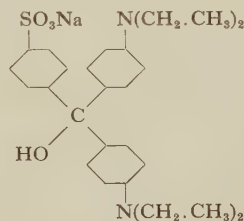
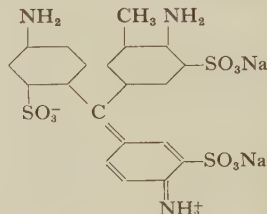
	First figure: 100 % germination Second figure: no germination	concentrations in p.p.m.	
		<i>Fusarium</i>	<i>Cercospora</i>
Bindschedler's green (chloride)		10-50	10-50
Brilliant cresyl blue (chloride)		10-50	1-10
Phenosafranine (chloride)		10-100	5-10
Methylene blue (chloride)		10-1000	—
Neutral red		10-100	10-50
Meldola's blue (chloride)		5-50	—

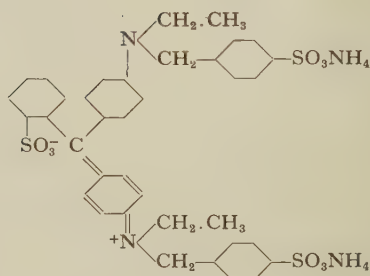
TABLE 4 (continued)

Phenyl-*p*-phenylene diamine
(chloride)*Fusarium*
50-500*Cercospora*
—TABLE 5. Substances failing to inhibit the germination of the spores of *Fusarium culmorum* at a concentration of 1000 p.p.m.*Tetraethyl-*p*-diamino-*p*-sulphonate
triphenyl carbinol

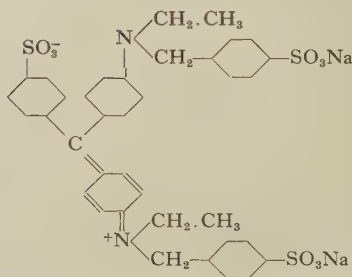
*Acid fuchsin



*Erioglaurine A



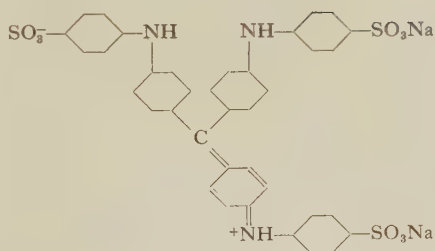
*Eriogreen SFL



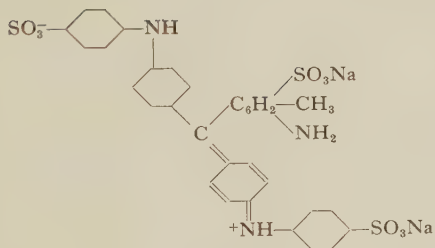
* These substances were all tested on the spores of *Cercospora herpotrichoides* and in all cases were found to be non-toxic at the highest concentration used (100 p.p.m.).

TABLE 5 (continued)

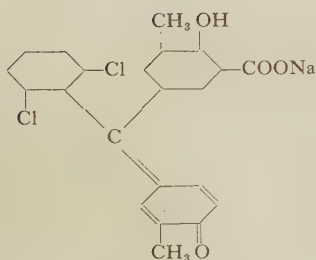
*Helvetia blue



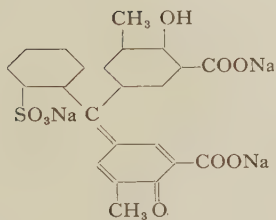
*Methyl blue (water-soluble)



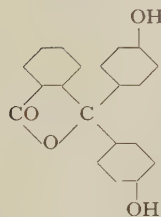
*Eriochrome azurol



*Eriochrome cyanine R



*Phenolphthalein (10 % alcohol in soln. 1000 p.p.m.). Also tested as the sodium salt



p-Aminobenzyl alcohol
Aniline hydrochloride
Dimethyl-*p*-toluidine
Leuco-malachite green

* See footnote, p. 228.

Notes on Tables 1-5

The substances were tested in the forms most easily available, usually as chlorides or bases, though occasionally as sulphates or some other salt (e.g. oxalate).

In order to ascertain whether the acid radicle had any influence upon toxicity a series of salts was prepared from the corresponding bases and comparative tests made (Table 1).

In all cases, within the bounds of the sensitivity of the experiment, the toxicity was unaffected by the acid used. For the sake of uniformity in the tables, therefore, the formulae of all basic substances used have been written as the base and not as the salt, but the acid radicle is expressed within brackets. In the case of acidic substances, however, the actual form tested has been given.

All substances which showed any signs of toxicity were observed to stain the spores, and in a few cases staining occurred before complete inhibition of growth; sometimes the hyphae were stained.

No non-toxic substances tried were seen to stain the spores, but in some cases they were observed to stimulate growth, e.g. Eriogreen SFL, Eriochrome Azurol, Eriochrome Cyanine and Tetraethyl-*p*-amino-*p*-sulphonate triphenyl carbinol, which actually stimulated the growth of the *Fusarium* hyphae so much that the mycelium was sporng after 24 hr., a phenomenon never observed with the controls.

DISCUSSION

Examination of the list of substances tested shows that *p*-amino derivatives of di- and triphenyl carbinol inhibit the growth of fungal spores, the efficiency increasing with the number of amino groups. Thus, among the triphenyl derivatives, dimethyl-amino triphenyl carbinol, malachite green (tetramethyl-diamino) and crystal violet (hexamethyl triamino), the effectiveness increases in that order, but the difference in toxic values between the di- and triamino compounds is slight.

When alkyl groups of increasing size are substituted in the amino group of a series of dyestuffs, the toxicity decreases with the size of alkyl groups (Table 1), but the positive influence of methyl and ethyl substituents in the amino group, although not great, is definite as is shown by comparing magenta with methyl violet 2B and the crystal violets. Victoria blue and night blue when compared with crystal violet show that the introduction of more complex groupings for the dimethyl aniline radicle effects no accentuation of toxicity.

That the toxicity is not due specifically to the central carbon atom is shown by the fact that its replacement by a nitrogen atom, although lowering toxicity, gives rise to relatively highly potent fungicides, as, for example, in Bindschedler's green and

the other series of substances in which the linking atom in the *para* position to the amino groups is nitrogen (Table 4).

The relatively high toxicity of substances in which the substituted benzene rings are fixed by a second bridging atom, as in acriflavine or brilliant cresyl blue, indicates that the toxicity does not depend in the free rotation of these rings.

The most marked effects upon the toxicity of these compounds arose with the substitution of acidic groups, e.g. carboxyl or sulphonic acid groups, e.g. in acid fuchsin and tetraethyl-*p*-diamino-*p*-sulphonate triphenyl carbinol, which are relatively non-toxic. The fungicidal properties of the dyes tested are limited to those having a basic reaction, except in the case of aurine, which, however, contains neither carboxyl nor sulphonic acid group (Table 5).

The slight toxicity of any of the acidic substances tested, the only one showing any toxicity being aurine, the reduction of growth by dimethyl-*p*-amino triphenyl carbinol, and the lack of toxicity of *p*-aminobenzyl alcohol, and *p*-toluidine, aniline and malachite green leuco base, suggest that toxicity is not a function of any one part but is due to the cumulative effect of the whole molecule.

Quastel (1931, 1932) and Quastel & Wheatley (1931) have shown that the basic dyestuffs of the triphenyl methane series and such colouring matters as auramine, acriflavine, safranin and methylene blue, inhibit the oxygen uptake of *B. coli*, in a number of media, whereas acidic dyestuffs of the triphenyl methane series (e.g. acid fuchsin and acid green) are either non-toxic or only very slightly toxic.

He suggests that the lethal action is due to combination with certain enzymes, which would result in the toxic dyestuffs staining cells, although the converse would not necessarily be true.

In the inhibition of fumarase, by dyestuffs, he found that acid dyestuffs of congo red series were most toxic, the basic dyestuffs of the triphenyl methane series being next in the order of toxicity, whilst the acid dyestuffs of this series were very much less toxic. Such dyestuffs as neutral red, safranin and methylene blue showed but low toxicity.

The action of dyestuffs on urease showed the acid dyestuffs to be non-toxic whereas the basic dyestuffs were toxic. But here the toxicity of the basic triphenyl methane dyestuffs were greatly enhanced by the presence of unsaturated glycerides.

In the action of dyestuffs on invertase it was observed by Quastel & Yates (1936-7) that the reduced dyestuffs (by hydrosulphite) showed very much less toxicity than the dyes themselves.

The parallelism of results in the inhibition of oxygen uptake in bacteria, in toxicity to enzymes and to fungal spores suggests that the mode of action of the dyestuffs is on the enzyme systems in the spores.

The staining of spores by toxic dyestuffs would further reinforce this view.

We wish to express our thanks to Messrs Geigy

and Co. Ltd., for a gift of certain of the dyestuffs tested, to Miss Mary D. Glynne for access to her private records, and also to Dr F. Tattersfield for help and advice in all stages of the work.

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Studies of fluctuations in insect populations

XI. The interrelationship of the wheat blossom midges and their host plant

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(With 2 Text-figures)

The date of wheat blossom midge emergence in an insectary some distance away from the growing wheat can give as reliable an estimate of the forwardness of the crop as field observation of the date of ear emergence.

The percentage grain attacked by the wheat blossom midges, which can be assessed by routine workers without any special experience of wheat, can give in early July a useful first estimate of the crop yield.

INTRODUCTION

In the eighth of these studies (Barnes, 1941) it was shown that the date of the emergence of the wheat-blossom midges (*Contarinia tritici* Kirby and *Sitodiplosis mosellana* Géhin) was correlated with the wheat-ear emergence and that both were correlated with the harvesting dates. It was also shown that the percentage grain attack was negatively correlated with the yield of wheat.

The purpose of this paper is to amplify these findings by additional data and to indicate their importance.

THE EMERGENCE OF THE MIDGES AND THE DATE OF HARVEST

In the previous paper it was stated: 'It should be possible to predict, in the first half of June, an early or late harvest, provided one knows the dates of either (the) midges' emergence or the 50 % ear emergence dates.'

In Table 1 the dates of emergence in the insectary of *Contarinia tritici*, and the dates of harvesting the wheat on Broadbalk are given for the period 1929-43. In addition, the dates of 50 % ear emergence in the wheat precision trial at Rotham-

sted 1933-9 are shown. The dates of emergence of the other wheat blossom midge, *Sitodiplosis mosellana*, are not given since they correspond very closely with those of *Contarinia tritici*. Reference to Tables 1 and 2 of the above-quoted paper, in which the weekly emergence of the two species for the years 1929-40 are given, will make this clear.

It can be seen that the peak emergence of the midges is closely connected with the ear emergence, and it would seem that those soil and weather conditions which are favourable to the one are also favourable to the other. In general, the earlier the ear emergence the earlier the harvest though the weather conditions between these two dates may cause considerable variation.

Thus, though the date of emergence of midges and of ears can only give a general indication of the earliness or lateness of the harvest, the date of midge

Broadbalk, the highest yields occurring in the years of low infestation and low yields in the years of high infestation.

The correlation between the actual Broadbalk yields and the percentage attacked grain is not significant, but when adjustments to the yields 1927-31 are made to allow for the differences in following,* the correlation practically reaches significance. These adjustments are based on the average yields of the various sections after the 1 year in 5 years fallowing system had settled down, so that it is likely that the adjustments made to the 1927 and 1928 yields are on the low side.

If the two years 1927 and 1936, in which the percentage attacked grain is open to considerable doubt, are left out the correlation is better than 1 % significant, one-half the yield variation being removed by the regression.

TABLE 1. Dates of emergence of *Contarinia tritici* and of harvesting, 1929-43

Year	Dates of 50 % ear emergence precision trial Rothamsted	Dates of <i>C. tritici</i> emergence in insectary		Date of harvest on Broadbalk
		First	Peak	
1929	—	17 June	20 June	9 Aug.
1930	—	6 "	15 "	16-18 "
1931	—	9 "	16 "	17-18 "
1932	—	12 "	22 "	9-10 "
1933	13 June	30 May	5-7 "	27-28 July
1934	23 "	12 June	16 "	2 Aug.
1935	25 "	6 "	24 "	7 "
1936	23 "	12 "	19-20 "	21 "
1937	est. 18 "	5 "	12 "	10 "
1938	21 "	6 "	14 "	5-11 "
1939	25 "	8-10 "	18 "	17-18 "
1940	—	31 May	6 "	6 "
1941	—	21 June	28 "	18-20 "
1942	—	16 "	23 "	14-15 "
1943	—	31 May	12 "	3-4 "

emergence obtained in an insectary can give as reliable an estimate of the forwardness of the crop as field observation of the date of ear emergence. The insectary used is at the Rothamsted Experimental Station laboratories and so at some distance away from the wheat on the farm.

LARVAL INFESTATION AND THE YIELD

The percentages of grain attacked by both the wheat-blossom midges in samples of 500 ears from Broadbalk for the years 1927-43 are given in Table 2, together with the yields of that field. In addition, the average yields of Great Britain for the period 1927-38 are shown. The estimated yields given are those based on the correlation with the midge infestation on Broadbalk in early July.

Table 2 shows clearly that there is an inverse relationship between the amount of midge infestation (percentage grain attacked) and the yield of

It can be pointed out that the degree of infestation is more closely correlated with the average English yield than with the Broadbalk yield.

Figs. 1 and 2 illustrate the actual yields of Broadbalk and Great Britain as compared with the yields as estimated from the midge infestation on Broadbalk early in July. It will be noticed that the general trends of the estimated yields follow the trends of the actual yields quite closely.

* The yields for Broadbalk are not strictly comparable. From 1932 onwards four sections of the field were cropped, these sections carrying the first, second, third and fourth crops after fallow.

In 1927 there had been no fallow for some years before. The crop in 1928 came after a 2-year fallow; in 1929 the crop was the second year after fallow; in 1930, three-fifths of the area was cropped after a fallow of 2 years and the other two-fifths was the third year after fallow. In 1931 three-quarters of the area was the second year after fallow and the other quarter the fourth year after fallow.

From this it would appear that the weather and soil conditions which control the intensity of the midge infestation are to a large extent also those which control the development of the wheat plant

according to the favourableness or unfavourableness of the weather.

The correlation between infestation and yield, which is controlled by the conditions in the 7-month

TABLE 2. *Percentage grain attacked by Contarinia tritici Kirby and Sitodiplosis mosellana Géhin and the actual and estimated yields of wheat on Broadbalk, together with those of Great Britain (cwt./acre)*

Year	% grain attacked	Yields (cwt./acre)			
		Broadbalk		Great Britain	
		Actual	Estimated	Actual	Estimated
1927*	(3.2)	9.8†	—	17.4	—
1928	6.5	24.0†	19.3	18.1	18.6
1929	7.7	11.9†	18.6	19.1	18.4
1930	17.6	14.0†	12.5	15.9	16.9
1931	21.4	12.5†	10.1	16.1	16.3
1932	15.8	11.2	13.6	17.2	17.2
1933	2.1	16.4	22.0	19.0	19.2
1934	4.0	22.0	20.9	19.9	18.9
1935	20.0	11.2	11.0	18.3	16.5
1936†	(9.7)	7.7	—	16.2	—
1937	15.6	9.5	13.7	16.1	17.2
1938	3.4	24.8	21.2	20.3	19.0
1939	9.8	16.3	17.3	—	—
1940	8.6	23.5	18.0	—	—
1941	11.3	13.4	16.4	—	—
1942	8.8	21.8	17.9	—	—
1943	10.0	21.3	17.1	—	—

* In 1927 the number of grains was counted in another sample taken $3\frac{1}{2}$ weeks after the sample in which the attacked grains were counted. In all other years the total number of grains was counted in the same sample as the number of attacked grain.

† Broadbalk yields for 1927–31 adjusted to allow for different fallow effects (see p. 232).

† In 1936 there was a very heavy thunderstorm (with over 3 in. rain) just when the period of peak emergence of midges was being approached, so that the figure for percentage attack obtained is undoubtedly too low.

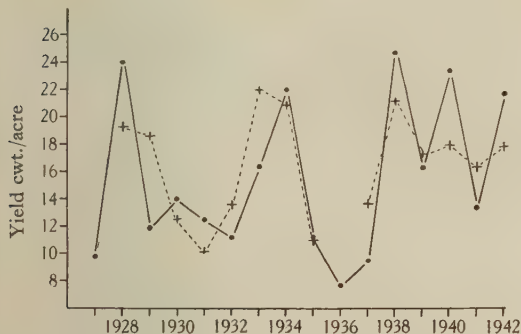


Fig. 1. Actual (full line) and estimated (dotted line) yields of wheat on Broadbalk

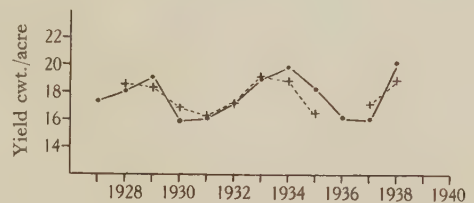


Fig. 2. Actual (full line) and estimated (dotted line) yields, Great Britain

during the 7 months between winter sowing and larval infestation (early July).

It can hardly be expected that the estimate based on the infestation in early July can give an accurate forecast of the yield a month later, since obviously local conditions in this month must affect the yield to some extent either beneficially or the reverse

period common to the life history of the midges and the wheat, removes half the variance of the yield as has already been stated. The remaining variance may be made up by the weather and other conditions during the last month in the life of the wheat after the larval infestation count and also by those factors during the infestation period in the previous late

summer prior to the sowing of the wheat. It was shown (Barnes, 1941) that the rise and fall in population of the midges could be affected in at least two ways during this period. For example, 'if the weather is unduly dry the fully grown larvae cannot leave the ears of wheat to pupate in the soil. Under such circumstances there is a chance that they will be removed with the crop from the field'. Again, 'In most years a partial emergence of *C. tritici* occurs in August and sometimes early in September. Whatever the factors are which cause this emergence they can greatly influence the population the next year in either direction, increase or decrease. As this supernumerary generation can breed successfully on couch grass, it is easy to see how a very large additional increase in the population can become available to attack the wheat the following year, if this partial emergence is considerable and conditions are suitable for these midges to reach the larval stage before the winter. On the other hand, if this summer generation is considerable but its G_1 cannot develop sufficiently before the winter sets in, it may materially reduce those available for the next year's infestation' (of the wheat).

Unless and until these factors can be evaluated the midge infestation can only give an early indication of the nature of the yield to be expected.

Where crop forecasts are required it is usual to start with an estimate of which it may be said that the earlier it is made the more of a guess it must be. A series of field observations will then adjust this first estimate so that the nearer to the harvest the

closer the estimate should be to the actual yield. It would be useful if this first estimate could be based on some measurable biological information, such as infestation by the larvae of the wheat blossom midges which can be assessed by routine workers with no special experience of wheat or crop forecasting.

From this study of wheat midges on Broadbalk (Barnes, 1941, 1932), it appears that Broadbalk is very suitable as a 'field laboratory' for measuring fluctuations of midge population; and these in their turn are an indication of those conditions favourable to the development of wheat. Among the reasons for this are apparently the facts that Broadbalk is undisturbed by rotation of crops and the midges have been present long enough for an equilibrium to have been set up between them and their parasites and other enemies. The absence of either of these conditions would result in violent fluctuations in population other than those caused by weather and soil conditions.

The estimates of yields based on larval midge infestation on Broadbalk show less fluctuations in the case of the yield of the whole country than in the case of the yield on Broadbalk itself. This suggests that on the one hand the midge population is controlled more by the major differences of weather, etc., from year to year and is less affected by the local variations of these major differences. On the other hand, it suggests that the development of the wheat plant on Broadbalk, while affected by these major differences of weather, etc., is also largely affected by the local variations on that field.

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Observations on the biology and physiology of wireworms of the genus *Agriotes* Esch.

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(With 10 Text-figures)

Detailed studies of the growth of wireworms kept at a constant temperature of 15° C. for 3 years and at natural soil temperatures for 2 years are presented. It is shown that small wireworms grow rapidly and moult frequently, but large wireworms grow slowly or remain constant in weight and moult infrequently. A basic pattern to account for the decreasing frequency of moulting during the life cycle is presented.

The feeding activity and moulting frequency of a population of wireworms has been studied in detail throughout the season. Definite peaks in feeding activity and moulting frequency occur during the season. A suitable method for the analysis of such activity in a population has been found by dividing the population into groups on a basis of the number of annual moults.

It is shown that increase in dry weight is a more valid estimate of growth than increase in fresh weight. The rate of growth of wireworms is strongly influenced by the food on which they feed: some plants (wheat and carrots) permit rapid growth; other plants (grass and clover) permit slower growth; while certain other plants (mustard and potatoes) allow the wireworm to maintain its weight. Wireworms reared on flax actually decrease in dry weight.

In a field experiment it was found that the type of crop grown after breaking up old pasture had a distinct effect on the wireworm damage done to the following cereal crop. Beans had the effect of reducing the population to a level at which a very successful crop was grown. Better yields were obtained after wheat and grass than after the other crops, excepting beans, at similar levels of population.

The cuticle of wireworms is permeable to water, and it is shown that in its relations to soil moisture the wireworm may be regarded as an osmotic system. The pF scale of soil moisture is shown to be of value in expressing the soil-moisture relations of wireworms. Wireworms feed more actively in moist soil than in dry or wet soil.

I. INTRODUCTION

The long life of the wireworm, 4, 5, or even more years, and the high mortality of the early stages have proved a great disadvantage for studying growth and very little work seems to have been published. Gueniat (1934) published a generalized graph based on his own few quantitative observations on *Agriotes lineatus*. He suggests that little growth occurs during the first 3 years, the wireworms weighing about 10 mg. at the end of this period. During the fourth year there is a very rapid increase in the rate of growth, an increase of 40 mg. being shown; during the fifth year growth is slower, and an increase of only 5 mg. is shown. Similarly, detailed evidence is lacking on the seasonal frequency of moulting. Gueniat also showed that between hatching and the end of autumn the larvae of the three *Agriotes* species generally moult twice, and also that larger individuals moult 1-3 times during the calendar year. Miles & Cohen (1941) state that there are generally two moults in the first few months after hatching and generally one moult each year afterwards. Several of the earlier investigators, Roberts (1919) and Langenbuch (1932), suggested two annual moults. Gueniat

(1934) summed up his observations in the following rather despairing statement: 'Dès que l'on s'attache à observer en détail un matériel sûrement déterminé, on est loin de trouver l'uniformité et la régularité que l'on pourrait attendre.'

Evans & Gough (1942) described the growth of various sized wireworms over periods of 25-40 weeks, and the results showed that the smallest wireworms studied grew most rapidly and moulted most frequently, and that the rate of growth and frequency of moulting declined as the wireworms increased in size. These results have now been largely confirmed and greatly extended. The observations described below suggest that there may be a basic plan in the seasonal frequency of moulting and also that deviations from it are, at least, partly explicable.

The wireworms used in this study have been obtained from the following sources. Those used in the studies on growth at constant temperature, on the effect of various foods on growth, and on the relationship between wireworms and soil moisture were taken from large samples sent in by nurserymen from infested glasshouses. Those used for the study of growth at natural soil temperatures and of population biology were collected in September 1941

from a field in its first year of arable cultivation (flax) after permanent grass. A large sample of soil was thoroughly examined and all the wireworms found (185) were placed individually in glass tubes, 3×1 in., containing moist soil and a grain of wheat. The glass tubes were kept in wire baskets placed in drain pipes sunk into the ground and were covered with another basket full of earth. The larvae were thus kept at 4–6 in. below surface level. The soil temperature at a depth of 4 in. was read daily at 9 a.m. B.S.T. or 10 a.m. B.D.S.T. First instar larvae were obtained from eggs laid in the laboratory by the three common species of click-beetles, *A. obscurus* L., *A. lineatus* L. and *A. sputator* L.

2. GROWTH STUDIES

(i) *The growth of individual larvae*

Fig. 1. shows some typical growth curves selected from those of the thirty-four surviving larvae of various sizes kept at a constant temperature for 3 years. The smallest larva (no. 1), weighed 1.1 mg. in Mar. 1941 and moulted three times between 1 May and 24 July inclusive. Growth continued until 4 Sept., by which time it had increased in weight by 11.5 mg. It then entered into a quiescent state for 3 months, although the temperature remained steady at 15° C. It fed again during the week 11–18 Dec. and then entered a moulting phase, moulting on 22 Jan. 1942. After a feeding period of 2½ months it entered another moulting phase and moulted on 30 Apr.; feeding then recommenced and ended on 23 July, when a second quiescent period began. During the growth period the wireworm increased in weight by 21 mg. The second quiescent period was terminated on 21 Jan. 1943 by a very short period of feeding followed by moulting on 4 Mar. Since this moult occurred, very little feeding has taken place, and the wireworm has increased in weight by 5 mg. (Dec. 1943).

Wireworm no. 2 weighed 5.9 mg. in Mar. 1941. It fed until 1 May when it entered a moulting period and moulted on 12 June. Then after a feeding period of 4 weeks it entered another moulting period and moulted on 21 Aug., after which date it fed intermittently for a time and then entered a quiescent phase for several months. During the period of active growth the wireworms increased in weight by 14–15 mg. This quiescent period was terminated in Apr. 1942 by a moulting period, and this was succeeded by another quiescent period until Jan. 1943 when a short period of active feeding commenced, followed in June by another moulting period. The second growth period resulted in an increase in weight of about 5 mg. and the third of about 14 mg.

Wireworms nos. 3 and 4 weighed approximately 53 mg. in Jan. 1941. During the 3 years' observations no. 3 has increased but slightly in weight

although it has moulted three times. No. 4 increased in weight appreciably during the first year of the observations, from 52.6 to 68.2 mg., but did not do so during the remaining 2 years except during the moulting periods. During the 3 years it moulted three times, and the last moult, 15 Apr. 1943, was followed by pupation on 22 July. Similar changes in weight occur on pupation as on moulting, except that in the few instances so far observed there is no further appreciable loss in weight after the skin has been cast as is often the case at moulting. The pupa remained fairly constant in weight for 5 weeks, but there was another big loss of weight, 15 mg., at emergence followed by another smaller loss of 5 mg. during the period when the adult cuticle was darkening and hardening. This individual proved to be an *A. lineatus* L. The moult occurring 3 months before pupation would appear to be exceptional, since it has not been observed in any other case during the present investigations. Gueniat (1934) also only recorded one case in seventeen observations.

Although these larvae have been kept under conditions of constant temperature suitable for feeding and growth during the whole period studied, the smaller larvae, initially 1–10 mg., show regular and distinct periods of inactivity lasting several months, alternating with periods of feeding and growth. These periods of inactivity would appear to correspond with the periods of hibernation shown by larvae kept under conditions of natural soil temperatures (see Fig. 2), but they tend to occur earlier. These inactive periods are not always clearly shown in the growth curves of larger larvae because the rate of growth is very much slower in the later stages of development, and some of these larvae hardly increase at all in weight during the growing season and some actually decrease in weight.

In the case of wireworms kept at natural soil temperatures for over 2 years, weekly records of the growth of fifty-six individuals and of the initial and final annual weights of another sixty are available for study. An analysis of the results is given in Table 1, and three growth curves chosen as typical on the basis of the analysis are given in Fig. 2. The curves begin in Sept. 1941 just at the end of the season's growth. During the winter of 1941–2 no growth occurred. Growth began again in April 1942, and the two smaller wireworms increased rapidly in weight but the largest grew much more slowly. During the growth season of 1942 the smallest wireworm moulted three times, the middle-sized wireworm twice and the largest only once. Growth ceased again during the winter of 1942–3 and recommenced in Mar. 1943. Again the two smaller wireworms increased in weight more rapidly than the largest one. During this growth season the smallest wireworm moulted twice and the two larger ones only once.

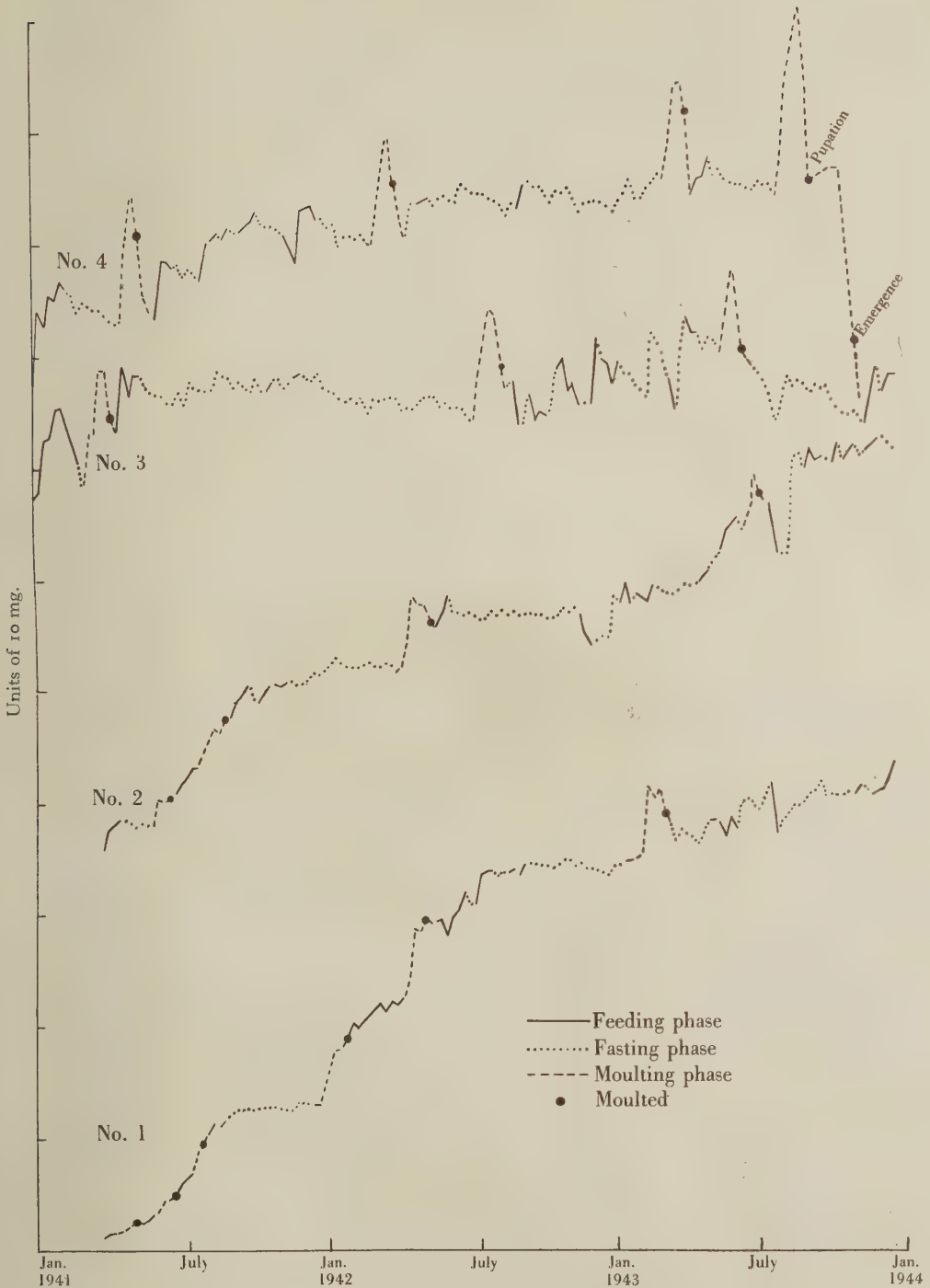


Fig. 1. Growth of wireworms at constant temperatures.

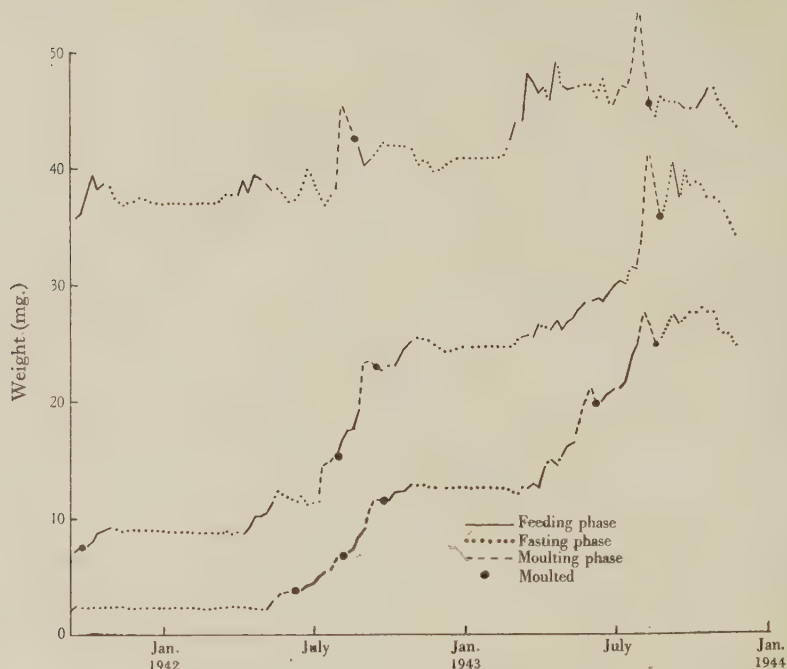


Fig. 2. Growth of wireworms at natural soil temperatures.

TABLE 1. Analysis of the results of 2 years' study on the growth and frequency of moulting of a population of wireworms. (Only those surviving for 2 years are included in the results)

A. Frequency of moulting

No. of individuals	1942 No. of moults	1943 No. of moults or pupation			
		3	2	1	P
21 (18 %)	3	0	15 (71 %)	6 (29 %)	0
57 (49 %)	2	1 (2 %)	15 (27 %)	38 (66 %)	3 (5 %)
38 (33 %)	1	0	3 (7 %)	22 (58 %)	13 (35 %)
116		1 (1 %)	33 (28 %)	66 (57 %)	16 (14 %)

B. Relation between frequency of moulting and growth

Moulting frequency		Initial wt. (mg.) 1942	Increase in fresh wt. (mg.) 1942	Increase in fresh wt. (mg.) 1943
1942	1943			
3	2	2.25 ± 0.35	9.53 ± 0.88	13.97 ± 1.01
	1	2.87	16.81 ± 1.40	10.67 ± 1.16
2	2	9.12 ± 0.65	11.53 ± 0.88	10.98 ± 1.59
	1	9.70	15.89 ± 0.63	6.16 ± 0.51
1	1	29.10	6.41 ± 0.93	3.31 ± 0.57
	Pupated	29.69 ± 1.13	6.65 ± 0.80	—
	2	29.23	5.5	6.1

(ii) Frequency of moulting in relation to size

Table 1 gives an analysis of the moulting behaviour and growth of 116 wireworms during 2 years based on their frequency of moulting during each year. Of the wireworms which moulted three times

in 1942, 71 % moulted twice and 29 % moulted once in 1943; of those which moulted twice in 1942, 2 % moulted three times, 27 % moulted twice, 66 % moulted once and 5 % pupated in 1943; of those which moulted once in 1942, 7 % moulted twice,

58 % moulted once and 35 % pupated. The second portion of Table 1 shows the relationship between rate of growth and moulting. It is clear that in the case of the 1942 three-moult group those which moulted once in 1943 had during 1942 grown much more rapidly than those which moulted twice in 1943. A similar difference is shown in the 1942 two-moult group; those which moulted twice in 1943 had not grown so rapidly in 1942 as those which moulted only once in 1943. In 1943, within both groups the increase in weight is greater for the larvae which moulted twice in 1943, thus bringing about some degree of compensation for slower growth in 1942. In the 1942 three-moult group those which moulted once in 1943 are still about 4 mg. heavier than those which moulted twice, but in the 1942 two-moult group compensation is complete. No difference in rate of growth of the 1942 one-moult group was observed between those wire-

A. lineatus moulted four times. The rates of growth, head width and proportions of individuals undergoing two or three moults are similar in *A. obscurus* and *A. lineatus*. The larvae of *A. sputator* do not seem to be as far advanced as those of the other two species, neither in frequency of moulting nor weight, also the head width is distinctly less in *A. sputator* than in the other two species. This is to be expected since *A. sputator* is much smaller than the other two species. Gueniat (1934) and Miles & Cohen (1941) have recorded two moults during the first growth season of the three species under consideration.

Few exact determinations of the length of the life cycle of wireworms of the genus *Agriotes* exist. Roberts (1921) obtained one adult *A. obscurus* after a life cycle of 5 years, and from the same brood a larva which had not yet pupated. Chrzanowski (1927) obtained five pupae of *A. obscurus* after a

TABLE 2. *Growth and moulting frequency during the first growth season*

No. of moults		1	2	3	4	Total or mean
<i>A. sputator</i>	No. of individuals	2	9	2	0	13
	%	15	70	15	0	—
	Mean wt. (mg.)	0.25	0.38	0.70	—	0.41
	Mean width of head (mm.)	0.250	0.292	0.370	—	—
<i>A. obscurus</i>	No. of individuals	0	13	7	0	20
	%	0	65	35	0	—
	Mean wt. (mg.)	—	0.55	0.71	—	0.61
	Mean width of head (mm.)	—	0.319	0.395	—	—
<i>A. lineatus</i>	No. of individuals	0	9	7	1	17
	%	0	53	41	6	—
	Mean wt. (mg.)	—	0.41	0.79	1.2	0.61
	Mean width of head (mm.)	—	0.327	0.406	0.500	—

worms which pupated in 1943 and those which moulted once again.

No definite age can be assigned to the larvae on which the above results were obtained. In order to correlate age, growth and moulting frequency, batches of eggs were obtained from *A. sputator*, *A. obscurus* and *A. lineatus*. Each newly hatched larva was isolated in moist soil in a small glass tube with a grain of wheat as food. The tubes were kept in wire baskets in drain pipes. Frequent examinations were made to determine the frequency of moulting, and all the exuvia found were mounted for further study. Most of the larvae hatched between 1 and 9 July 1943. At the end of November the survivors were weighed and the breadth of their head capsules measured. The observations were started on fifty larvae of each species, but by the end of November a 66 % mortality had occurred (see Table 2).

Table 2 shows that, at least under the conditions of the present experiment, about one-third of the total of *A. obscurus* and *A. lineatus* have moulted three times during this period. One individual of

4-year cycle with two individuals still in the larval stage. Thus it would appear from this meagre data that the life cycle might last from 4 to 6 years.

Although much intensive study is still needed before an adequate idea of the life cycle of *Agriotes* spp. is obtained, it is possible tentatively, on a basis of the above facts, to postulate a basic pattern for the annual moulting frequency of the larvae of *A. obscurus*. During the first growth season, the majority moult twice, during the second three times, during the third twice, during the fourth and fifth once each. At the end of the sixth growth season, pupation occurs. This gives a life cycle of 5 years from the egg to the newly emerged adult. From this basic pattern a 4-year cycle can be obtained by the elimination of the moult in the fifth growth season or else those larvae which moult three times in the first growth season could moult twice in the second season, once in the third and fourth seasons and pupate in the fifth. A 6-year cycle could be obtained by the interpolation of a growth season with either one or two moults.

3. POPULATION BIOLOGY

It has been possible to study the seasonal changes in feeding activity, growth and incidence of moulting in a population as compared with that of individual wireworms described above from the weekly observations on 175 wireworms kept under conditions of natural soil temperatures for over 2 years.

After the first winter, during which ten individuals died, the distribution of the population in Mar. 1942 in relation to weight is shown in the following set of figures (in mg.):

0-2	2.1-5	5.1-10	10.1-15	15.1-20
16	25	32	23	13
20.1-25	25.1-30	30.1-35	35.1-40	40.1+
12	17	18	13	6

The mortality during the 2 years' observations was comparatively low, being 15 % during 1942 and 9 % during 1943.

Feeding commenced in Mar. 1942 when the soil temperature at 4 in. depth rose to 5° C. The amount of food consumed rapidly increased to a maximum in April and remained at this maximum during April and the first week in May (Fig. 3A). During May, the amount of food consumed declined rapidly and was at a minimum during June. It then increased again during July and remained fairly high with three minor peaks of consumption until October, when it declined again and finally ceased during November. Evidence will be presented later in the section dealing with the relationship between wireworms and soil moisture that the method of expressing food consumption as a fraction of the wheat grain destroyed is a valid one.

The percentage of wireworms feeding increased to a maximum of just over 80 % in April and fell to nearly 10 % in June. During the period July-September it remained at a figure varying between 30 and 50 %. This figure is surprisingly low and would appear to be due to the relatively small and infrequent periods spent in feeding by large wireworms.

Moulting began towards the end of May, and during June as many as 10-12 % of the wireworms moulted each week. During July moulting was not so frequent and reached its maximum frequency of 15-20 % per week during August, after which it declined and finally ceased in October (Fig. 3B). Pupation occurred from mid-July to mid-August, and the adults emerged during August and early September. The periods of pupation and emergence correspond with those given by Miles & Cohen (1941) for field conditions.

During 1942 some of the wireworms moulted three times, others twice, others once, and some pupated. A few were parasitized, probably by the proctotrupid *Paracodrus apterogynous* Hal. The per-

centages of the various moulting groups are as follows:

3 moults	2 moults	1 moult
21	66	42
12.9 %	37.7 %	24.0 %
Pupated	Parasitized	Died
14	5	27
8.0 %	2.9 %	15.4 %

A suitable method for analysing the behaviour of the population has been found on the basis of the annual moulting frequency. The amounts of food consumed, moulting periods, etc., have been worked out for wireworms moulting three times, twice, once and pupation during the year, and the results are given in Fig. 3 C, D. The amounts of food consumed by the three-moult groups was very small during the first and second feeding periods but became appreciable during the third and fourth feeding periods. In the two-moult group, three definite feeding periods are apparent, during the first of which less food was consumed than during the third and fourth. The one-moult group has two feeding periods, but in this group feeding was much more intense during the first than during the second. The group which pupated had only one feeding period. The first feeding period was coincident in all four groups and clearly accounts for the intense feeding during April shown by the total population (Fig. 3 A, D). The long second feeding period from July to October shown by the total population is made up of the second feeding period of the one-moult group, the second and third of the two-moult group and the third and fourth of the three-moult group. The peak during July is principally due to the second feeding period of the two-moult group, and the second peak, in September and October, to the second feeding period of the one-moult group, the third feeding period of the two-moult group and to the third and fourth periods of the three-moult group. It is not thought that the decrease in feeding shown at the end of September is a significant one, since in the 1943 results only one peak is shown at this time of the year.

The time of year at which the various groups moulted varied considerably. The three-moult group moulted principally during June, August and September-October, the two-moult group during June-July and August-September, and the one-moult group during July-August (Fig. 3 C).

Rapid growth occurred principally in the two and three-moult groups. The two-moult group increased by an average of 14 mg. and the three-moult group by an average of 12 mg. during the year. The one-moult group increased in weight by an average of only 6 mg., and those which pupated by an average of only 3 mg. before the beginning of water absorption preparatory to pupation.

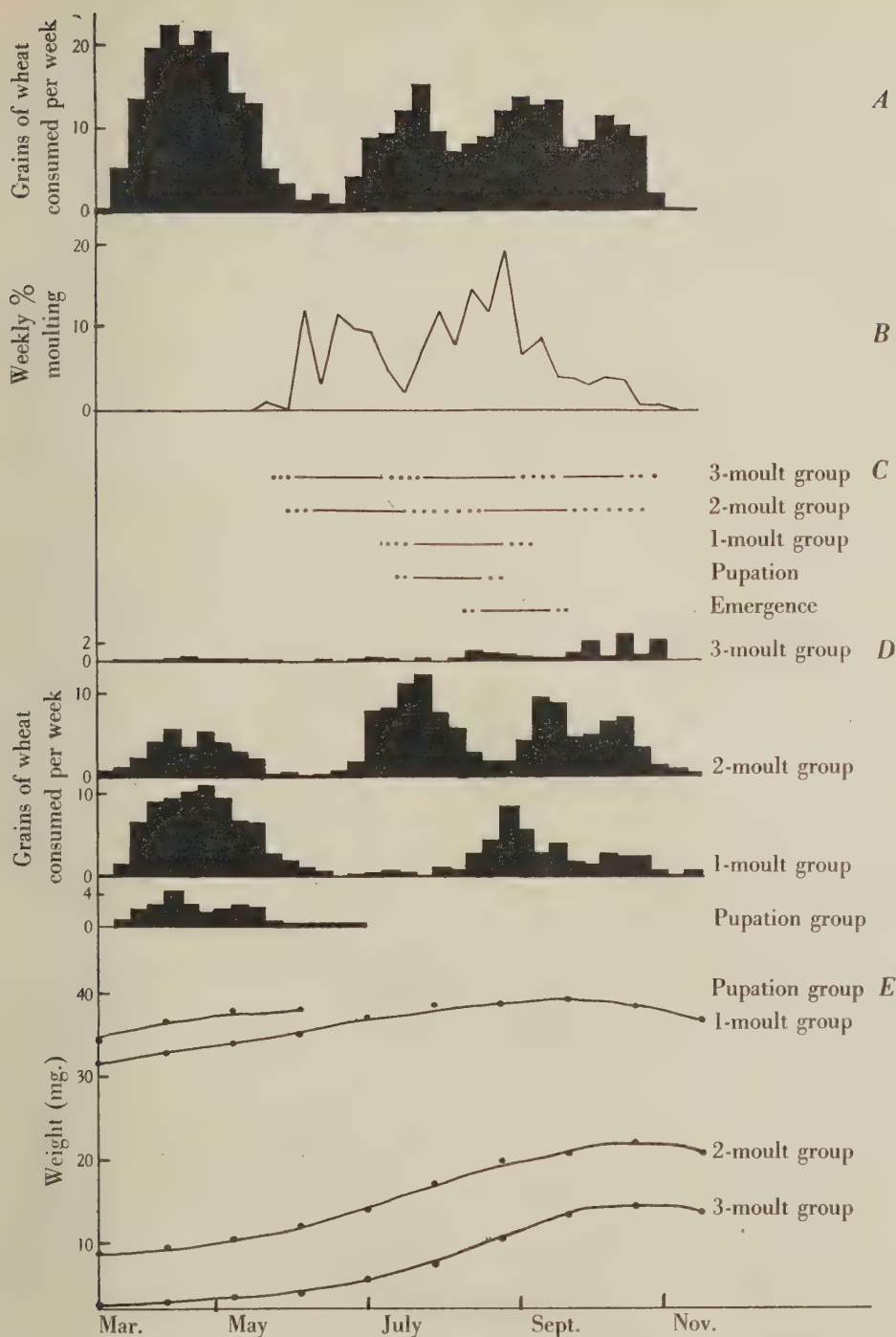


Fig. 3. The seasonal variations in activity of a population of wireworms during 1942. A, food consumption of the total population; B, moulting frequency of the total population; C, moulting periods of the several moulting groups; D, food consumption of the several moulting groups; E, growth of the several moulting groups.

Very similar results were obtained on the surviving wireworms during 1943. A three-moult group was absent during this year. The chief difference between the results obtained in 1942 and 1943 lay in the time at which the peaks of the various activities occurred. In 1942 the spring peak of feeding activity lasted during April and the first week of May, in 1943 it was just 2 weeks earlier; the July 1943 peak also was advanced by a similar period, but the September-October peak took place at approximately the same time. The spring moult of the two-moult group was earlier in 1943 than in 1942, but the autumn moult of this group and the moult of the one-moult group took place at about the same time as in 1942. The mean weekly temperature of the soil rose to 5° C. just 2 weeks earlier in 1943 than in 1942.

Cessation of feeding at the end of autumn does not seem to be associated to any great extent with any external factor such as temperature. Most of the wireworms ceased to feed by the end of October when the soil temperature was still well above 5° C. Only a few wireworms were still feeding when the soil temperature dropped to 5° C. and low temperature became a limiting factor. In view of the long cessation of feeding shown at the end of a period of growth by wireworms kept at a constant temperature of 15° C., it would appear that hibernation is, at least to some extent, a type of diapause.

These results have been obtained on a population whose size distribution is rather different from that of a normal field population with a predominance of small larvae, especially those of the three-moult group and the smaller ones of the two-moult group. The effect of a larger number of the small larvae would principally be to increase the amount of feeding during the summer and autumn feeding period. In view of the lack of knowledge concerning the proportions of the various size groups in field populations and the mortality in the groups during the year, it is not possible to speculate on the relative amounts of feeding shown during the year by such a normal population. The results are presented here chiefly to show that a technique is available for studying the seasonal behaviour of a wireworm population.

4. THE EFFECTS OF VARIOUS FOODS ON GROWTH

Evans & Gough (1942) showed that the increase in fresh weight of wireworms reared on wheat grains, carrot disks and bean seeds varied according to the food, being greatest on wheat grains and least on bean seeds. This work has been extended and the results are given in Table 3. Batches of twenty larvae, initially 20–25 mg. in weight, were fed for 21 weeks on wheat grains, carrot disks, bean seeds (*Phaseolus vulgaris*) and potato disks, and twenty larvae were also kept without food. The larvae were

confined individually in 3 × 1 in. glass tubes and were given fresh food every week. The experiment was carried out at a constant temperature of 15° C. During the course of the experiment all the larvae moulted once or twice. The initial dry weights of twenty larvae were determined.

TABLE 3. *Growth of wireworms fed on various foods*

		Initial fresh wt. (mg.)	Initial dry wt. (mg.)
		23.30 ± 0.40	11.91 ± 0.34
	No. surviving	Final fresh wt. (mg.)	Final dry wt. (mg.)
Wheat	12	37.25 ± 1.67	19.39 ± 0.96
Carrots	15	32.67 ± 1.02	18.30 ± 0.55
Beans	17	31.77 ± 1.14	12.47 ± 0.75
Potatoes	17	29.71 ± 1.54	12.05 ± 0.79
Starving	15	23.91 ± 0.71	5.05 ± 0.38

Wireworms fed on wheat grains increased most in weight on a fresh-weight basis, those fed on carrots, beans and potatoes increased in weight to a similar degree, while the starving wireworms remained constant in weight. On a dry-weight basis the results are different. Wireworms fed on wheat grains and carrot increased significantly in weight, those fed on beans and potatoes did not increase significantly, and those which were starved decreased significantly in weight. The various kinds of food did not appear to have any effect on the number of moults during the experiments, 40–50 % of the wireworms moulting twice, the rest once. Even the wireworms which were starved showed similar proportions in the numbers moulting once or twice.

Another experiment was carried out on the effects of various kinds of crop plants on the rate of growth of wireworms. Various crops were grown in wooden boxes 10 × 8 × 8 in., kept in a glasshouse. Five wireworms initially weighing 20–25 mg. were placed in each box and five boxes of each crop plant were grown. The experiment lasted from 19 Mar. to 4 Aug. 1941.

Wireworms reared on wheat and mustard increased in fresh weight significantly more than those reared on other crops, and those reared on flax significantly less than the others (Table 4).

However, on a basis of dry weight, the order of the effect of the crops on growth has changed considerably. The crops can conveniently be divided into four groups. Wireworms reared on wheat and carrot have increased significantly more in dry weight than those reared on the crops of groups 2, 3 and 4, and those reared on onion significantly more than those of groups 3 and 4. The members of group 2 have increased significantly more than those of groups 3 and 4. Wireworms reared on beans and mustard have not increased in weight signifi-

fantly, while those reared on flax have actually decreased in dry weight.

TABLE 4. *The effects of various crops on the growth of wireworms on a fresh-weight basis*

Crop	No. surviving	Final fresh wt. (mg.)
Wheat	11	35.98 ± 0.51
Mustard	15	35.31 ± 0.37
Grass	14	33.70 ± 0.40
Onion	9	33.68 ± 0.62
Carrot	10	33.51 ± 0.56
Clover	13	32.91 ± 0.44
Beans	18	31.93 ± 0.31
Flax	9	29.27 ± 0.62
Original fresh wt.		24.25 ± 0.35

TABLE 5. *The effects of various crops on the growth of wireworms on a dry-weight basis*

Crop	Final dry wt. (mg.)	Group
Wheat	13.71 ± 0.21	1
Carrot	13.42 ± 0.24	
Onion	13.30 ± 0.27	
Clover	12.80 ± 0.18	2
Grass	12.73 ± 0.17	
Beans	11.85 ± 0.13	3
Mustard	11.87 ± 0.16	
Flax	9.22 ± 0.21	4
Original dry wt.	11.47 ± 0.15	

It has frequently been stated in the literature that during the first year after ploughing up old grass, the wireworms feed mainly on the decaying turf. Evans & Gough (1942) showed that although wireworms confined in soil containing several types of decaying organic matter increased in fresh weight, they actually decreased in dry weight. The value of rotting turf was therefore tested as a source of nutrient for wireworms. Circles of turf, 3 in. in diameter, were cut from an old pasture. The following treatments were used: (1) whole turf buried; (2) all the green vegetation was cut off as closely as possible to the surface of the soil and buried; (3) the roots left over from treatment 2 were buried; (4) wheat grains provided as a source of food for control.

Fifteen replicates of each treatment were set up in 2 lb. jam-jars and one wireworm placed in each replicate. The experiment was carried out at 15° C. from 16 Sept. 1941 to 29 Apr. 1942.

Table 6 shows that the wireworms gained in fresh weight to an equal amount in all treatments. They gained appreciably in dry weight in all treatments; no differences obtained between the three turf treatments, but wireworms fed on wheat gained much more than those fed on turf. The turf had rotted well by the end of the experiment except for

the bulbous portion and basal portion of the coarse stemmed grasses. The unrotted portions were still being attacked at the end of the experiment. It is therefore possible that the value of buried turf as a source of food for wireworms may depend largely on the type of pasture, especially on the portion of the flora which most resists decomposition.

TABLE 6. *The value of rotting turf as food for wireworms*

	Fresh wt. (mg.)	Dry wt. (mg.)	No. surviving
Wheat	33.74	16.61	10
Top turf	33.80	12.22	12
Roots	32.60	12.79	13
Whole turf	33.35	12.22	14
Original	29.37	9.42	—

It is evident from the results given above that the value of different crops as a source of nourishment for wireworms varies considerably and may have a great effect on their subsequent behaviour. This is borne out by the results of an experiment on the cropping of newly ploughed grassland at Rothamsted. The relevant details of the experiment are as follows:

There were four randomized blocks of eight plots, one for each crop, each plot being split into four for fertilizer treatment. Four plots were assigned at random to each of the eight crops, winter wheat, beans (winter-sown failed, resown in spring), oats, barley, flax, sugar-beet, potatoes and hay. Each plot was $\frac{1}{16}$ acre in area.

The field was ploughed (except hay plots) in Oct. 1940 and lime (20 cwt./acre) applied at the end of November. All combinations of nitrogen, phosphorus, potassium and agricultural salt occurred for each treatment and also a control unmanured plot.

During the first year, some wireworm damage occurred on the cereal plots, and a determination of the population of the experimental area was carried out by the hand-sorting method. A population of approximately 750,000/acre was found; 128 samples yielded 191 wireworms or 1.5 wireworms per 4 in. core. In view of the variable effects of different crops on the growth of wireworms, it was thought that this experiment might show the effects of variable nutrition in one year on the amount of damage done to the following crop of spring oats. The spring oats crop was sown at the end of Mar. 1942. By the end of April it was clear that the amount of damage being done by wireworms varied greatly on the different plots. Plant counts were carried out on 4, 18 and 19 May. On each subplot the numbers of plants on five 1 yd. strips taken at random were determined (Table 7).

The plant counts clearly fall into three groups. On those plots which carried beans in 1941, an excellent

stand was produced. On all the other plots wireworm damage was clearly marked, but the damage was less marked on the plots formerly carrying wheat or grass than on the other plots. As a result of these observations, the wireworm population of the various plots was estimated in June. Approximately the same population was found as in 1941, but a marked exception was found in the population of the plots which formerly carried beans. On these plots there had been a great reduction in the wireworm population of about 75 %.

The yields of grain also fall into three groups. The maximum yield was obtained on the plots formerly carrying beans and was clearly due to the great reduction in the wireworm population from a dangerous level to one at which successful cereal crops might be expected (Anon. 1941). The plots formerly carrying wheat and grass yielded distinctly better than the crops listed below them in Table 7. It is considered that this is very possibly due to the

TABLE 7. *Variation in oat plant and wireworm populations following various crops*

Previous crop 1941	Oat plant numbers 1942, 1000's per acre		Wireworms 1000's per acre		Crop yields 1942 cwt./acre
	1st count	2nd count	May 1941	June 1942	
Beans	1110	780	950	225	28.5
Grass	650	460	550	650	19.4
Wheat	620	430	725	725	18.7
Sugar beet	590	360	875	625	14.9
Potatoes	580	360	775	650	9.8
Barley	550	330	750	900	14.8
Flax	520	290	800	725	12.2
Oats	490	310	525	550	9.6

better conditions of nutrition provided in the previous year by wheat and grass than by the other crops, two of which, potatoes and flax, have been shown (Tables 3, 4) to be poor sources of nourishment.

The reason for the marked decline in population following the growing of a bean crop is not known. There appear to be two possibilities: first, a migration of the wireworms away from the bean plots, which in this experiment are small (110 × 28 ft.), secondly, some kind of toxic effect by the roots of the bean plants on the wireworms. The first explanation seems unlikely to be correct, since the wireworm sampling carried out in May 1941 indicated a high population on the bean plots, whereas, if migration were going to occur, it is likely that it would have started by that time. An attempt was made to investigate this possibility, in 1942, by intensive sampling of the bean plots and plots adjacent to them. The results did not give any indication of an increased population on the borders of the adjacent plots, but an excessive number of

samples would have been required to give a clear result. Similar big reductions in wireworm populations have been recorded in certain cases, under normal field conditions, which would preclude any migration except around the edges of the field (Finney, 1943).

5. SOIL-MOISTURE RELATIONS

(i) *The permeability of the cuticle to water*

Evans & Gough (1942) described in detail the complex changes in weight associated with moulting in the wireworm. Small wireworms, initially 1–5 mg. in weight, continued to increase in weight although they had ceased to feed. After fasting for a period of about 2 weeks they moulted and still continued to increase in weight for approximately a week before beginning to feed again. Larger wireworms, initially 20–25 mg. in weight, decreased in weight slightly when they ceased to feed preparatory to moulting. After a fasting period of 5–8 weeks their weight increased by 15–20 %. Moulting occurred after a decrease in weight of 0–20 %. These results were obtained in Rothamsted Allotment soil with a moisture content of approximately 22 %, and it was assumed that the increase in weight preparatory to moulting was brought about by the wireworms drinking water present in the soil.

An experiment was carried out to see if moulting was inhibited by dry soil. Fourteen large wireworms were confined in soil with a moisture content of 10 % in glass tubes (5 × 1½ in.). The soil at this moisture content was very dry to the touch, and it seemed unlikely that wireworms could obtain any water by drinking. Contrary to expectation the wireworms moulted and showed the typical changes in weight associated with moulting. Since it seemed so unlikely that water was obtained by drinking in this case, an experiment was carried out to see whether wireworms in the premoulting phase could increase in weight when physically prevented from drinking. This was done by coating the head with paraffin wax and also by tying a ligature between the 1st and 2nd thoracic segments. Wireworms submitted to both treatments all showed the typical increase in weight preparatory to moulting. Thus it is evident that this increase in weight is brought about by an absorption of water through the cuticle. This permeability of the wireworm cuticle to water has previously been noted by Subklew (1934) and Woodworth (1939).

(ii) *The effect of solutions of varying osmotic pressure on wireworms*

In order to study further the absorption of water by wireworms, a preliminary experiment was carried out by immersing wireworms varying in size in

* Expressed as a percentage of the dry soil.

different concentrations of potassium chloride. Three groups were used, small normal 9–15 mg., large normal 35–45 mg., and large premoulting phase 45–50 mg. They were weighed, placed individually in glass vessels containing 0, 1, 2 and 5 % potassium chloride. They were weighed again after 2½ days' immersion at 20°. Fig. 4 shows the results obtained. The most striking result is the difference in shape of the curves obtained for wireworms in the premoulting phase and normal individuals. The former show a progressive and regular increase in weight with decreasing concentration, the latter show a pronounced flattening of the curve between 0 and 1 % concentration. Most of the wireworms used were alive at the end of the experiment, so that the results cannot be attributed to post-mortem changes. A few deaths occurred in the premoulting phase

to be inhibited, and the wireworm absorbs water until its cuticle is stretched to a maximum volume or it may even burst. This inhibition of excretion in the premoulting phase may be due to the histological changes associated with the renewal of the cuticular lining of the hind-gut.

Proof of the correctness of the above hypothesis can be obtained by ligaturing a wireworm between the 1st and 2nd thoracic segments and between the 8th and 9th abdominal segments and immersing it in distilled water. These ligatures prevent drinking and excretion, and in 3–4 days the wireworm will be fully expanded, having increased in weight by 30–40 %. As a first approximation it would seem that in relation to the surrounding liquid the wireworm may be regarded as an osmotic system, being bounded by a semipermeable membrane, the cuticle and/or hypoderm. Thus should the surrounding liquid be hypotonic to the blood the wireworm will absorb water, but should it be hypertonic the wireworm will lose water.

An experiment was carried out to test the degree to which the above supposition holds. Woodworth (1939) showed that the cuticle of *Limonius* spp. is permeable to arsenite ions. In the present experiment, solutions of sucrose were used instead of potassium chloride as its molecules are much larger and so there is less chance of any penetration into the wireworm. Ten replications were carried out at each of seven concentrations, one series with normal wireworms and another with anaesthetized wireworms in order to inhibit excretion. Anaesthesia was carried out by adding 2 ml. of pure ethyl ether to 300 ml. of the sucrose solution. Any osmotic effect of this concentration of ether has been neglected, since even if none was absorbed by the wireworm its osmotic effect would only be equivalent to that of a 0.007 *M* solution of sucrose. The wireworms were weighed daily in order to determine whether equilibrium was reached at any of the concentrations. The experiment was continued for 7 days only, as many of the wireworms had died at the higher concentrations by this time.

There are certain definite differences between the results obtained on anaesthetized individuals and on normal individuals (Fig. 5). The most noticeable difference is shown in the 0.0 and 0.15 *M* solutions. Normal wireworms increase slightly in weight for the first 3 days and then remain fairly constant in weight; anaesthetized wireworms, however, increase in weight during the 7 days of the experiment. Another interesting difference shown is the greater loss of weight, during the first 3 days, of anaesthetized wireworms in 0.45 *M*. There are no significant differences in the loss of weight of the two series at 0.60 and 0.75 *M*. The effects of immersing normal wireworms in sucrose solutions are twofold, osmotic and drinking. Normal wireworms immersed in coloured solutions of water or sucrose can drink it

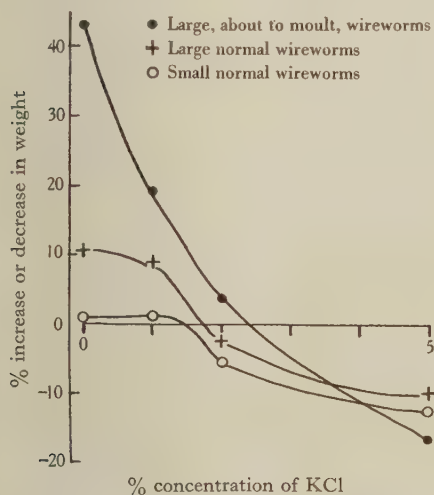


Fig. 4. The effect of varying concentrations of potassium chloride on the weight of wireworms.

group immersed in distilled water. Death was caused by rupture of the cuticle due to the great internal pressure.

The simplest hypothesis which can be advanced to explain these results is that in normal wireworms a regulatory mechanism is present which gets rid of a certain amount of the absorbed water. This mechanism is clearly the normal excretory system, malpighian tubules and hind intestine. The small larvae do not increase significantly in weight in 0 and 1 % potassium chloride, so that their excretory system is able to dispose of the absorbed water. The large normal larvae increase in weight by about 11 %, so it appears that they can only excrete that amount of water absorbed after the osmotic pressure of their blood has been reduced by dilution. In the case of large wireworms in the premoulting phase, regulation through the excretory system would seem

as is shown by the presence in some cases of coloured liquid in the gut on dissection. This explains the increase in weight of normal wireworms in 0.0 , 0.15 and $0.30M$ solutions and also the greater loss of weight of anaesthetized wireworms at $0.45M$, since these wireworms were unable to drink.

Unfortunately, the wireworms did not reach equilibrium at several of the concentrations used, as they died, due to rapid loss of water at the higher

difference between the effect of 0.0 and $0.15M$ solutions on normal and anaesthetized wireworms; the mean increase in weight of the normal wireworms was 8 %, but that of anaesthetized wireworms was 35 % at $0.0M$ and 22 % at $0.15M$. It is evident, therefore, that normal wireworms excrete most of the water which they absorb when immersed in hypotonic solutions.

The general resemblance between the curves

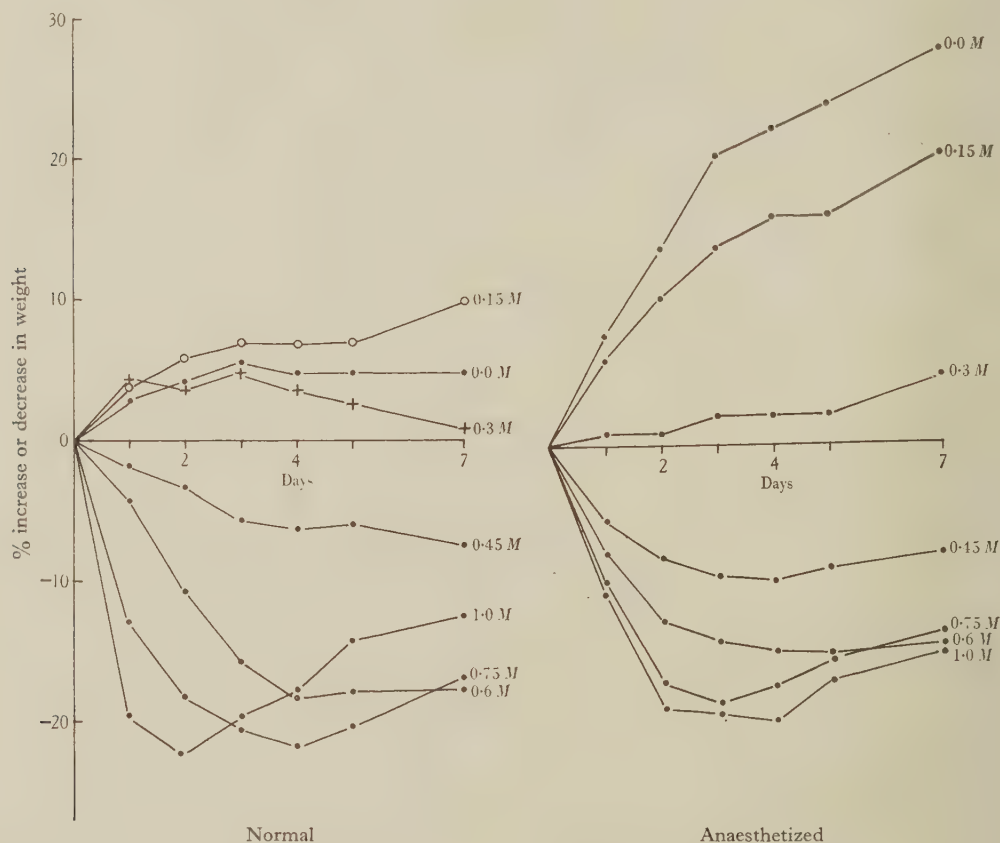


Fig. 5. The effect of varying concentrations of sucrose on the weight of normal and anaesthetized wireworms.

concentrations, so the experiment was terminated before equilibrium was reached at the lower concentrations. Fig. 6 has therefore been drawn up by plotting the maximum percentage gain or loss at any concentration against that concentration, thus eliminating to some extent post-mortem effects. The graph shows that there is little difference between the effect of various hypertonic solutions on normal and anaesthetized wireworms, neither is there any difference at the approximately isotonic concentration of $0.30M$. There is, however, a great

shown in Figs. 4 and 6 is evident. The same difference in the shape of the curves is shown between normal and premoult phase wireworms as between normal and anaesthetized wireworms. The chief difference lies in the isotonic values for anaesthetized and premoult phase larvae; the isotonic value for anaesthetized larvae is approximately $0.33M$, but that for the premoult phase larvae is approximately $0.60M$, nearly double. The isotonic value for normal larvae is approximately equal to that of anaesthetized normal larvae. The

greater isotonic value for larvae in the premoult phase is probably brought about by the liberation of products of histolysis into the blood. Wireworms in the premoult phase are much less active than normal wireworms, and the greater isotonic value for this stage will have the effect of increasing resistance to desiccation.

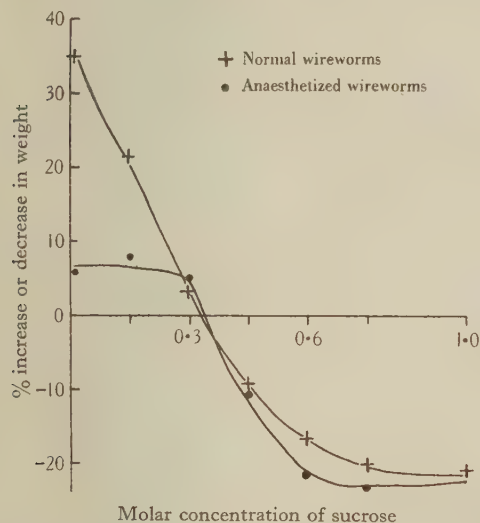


Fig. 6. Comparison of the weights of normal and anaesthetized wireworms in hypotonic and hypertonic solutions of sucrose.

(iii) *The effect of various contents of soil moisture on the weight of feeding and starving wireworms*

An experiment on the effect of twelve different contents of soil moisture on the weight of feeding and starving wireworms and also on the amount of food consumed was carried out. Batches of soil (a sterilized potting soil) differing in water content from 5 to 35 % were made up in bulk. The higher soil moistures, 50–75 %, could not be made up in bulk as the soil became too sticky to handle; therefore each tube was made up separately 2 days before it was required. A given amount of soil of known water content was weighed into the tube and the amount of water necessary to bring it to the required water content was added, the tube then being sealed with a waxed cork. Ten replications of starving and feeding wireworms were set up at each of the twelve soil-moisture contents. The wireworms were weighed at the beginning of the experiment and at the 1st, 2nd, 3rd, 5th and 7th weeks. The food used was wheat grains; these were examined and fresh grains added at the end of each week, the amount consumed being estimated as a fraction of a whole grain. The estimation was made by eye and was subject to the errors associated with this type of estimation,

but since the total amount consumed by the wireworms at any one soil-moisture content during the experiment would depend on seventy such estimations, it was concluded that the final error would not be large enough to hide any big differences. Evidence to justify this conclusion will be presented later. The pF at various moisture contents of this soil was determined on the drying curve, since it was found that during the course of the experiment the soil dried out slightly.

The final changes in weight for both series at the various soil moisture contents are shown in Fig. 7.

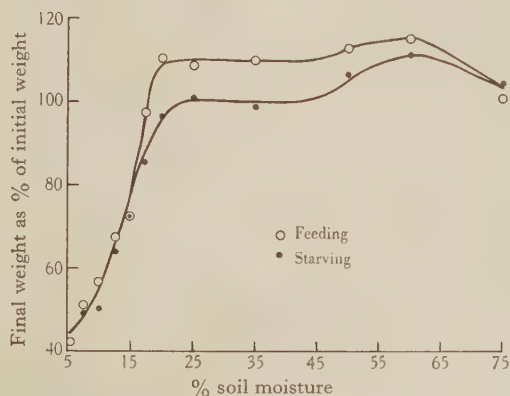


Fig. 7. The effects of varying soil-moisture content on the weights of feeding and starving wireworms.

The shape of the two curves is very similar. As the moisture content increases from 5 to 15 % the loss of weight decreases equally for both series; no food is eaten below 15 % soil moisture and only a small amount at this moisture content. From 15 to 22 % the weight lost is greater for the starving wireworms than for those provided with food. From 22½ to 45 % the starving wireworms remain constant at their original weight; the feeding wireworms also are constant in weight but at a level of 10 % above their original weight. At soil-moisture contents above 45 % the starving wireworms increase in weight appreciably. A similar tendency is shown by the feeding wireworms. Why there should be such a small increase at 75 % soil moisture cannot yet be explained. It may be associated with the lack of mobility of wireworms in saturated soils. Fig. 8 shows the weekly changes in weight of starving wireworms at the twelve moisture contents. The shapes of the various curves are very similar to those shown in Fig. 5 for wireworms immersed in iso- and hypertonic concentrations of sucrose. It is interesting to note that when the rate of loss of water is rapid (immersion in sucrose) death occurs when the wireworm has lost 20–22 % of its original weight (Fig. 5), but when the rate of loss is much slower

(in soil) the wireworm can withstand the much greater loss of 35 % at 12½ % soil moisture. The time of death could not be ascertained with great accuracy in the soil experiment, so an internal comparison cannot be made within the experiment. The amount of food consumed by the ten wireworms at each soil moisture is shown in Fig. 9. No food is consumed below a soil moisture of 12½ % (*pF* 4.8). Above this value the amount consumed increased rapidly to a maximum at 25–35 % and then decreased, reaching a low level at 75 % soil moisture. The varying water content of the grain at the different moisture contents had no effect on the estimation of the amount consumed, since this was estimated as a fraction of the whole grain.

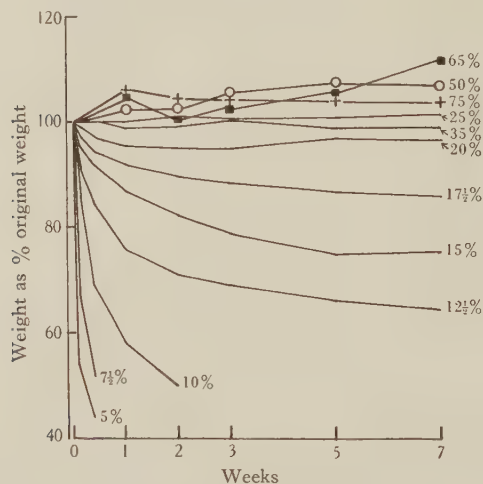


Fig. 8. Weekly changes in weight of starving wireworms at various soil moistures.

Lees (1943) has also studied the effect of moisture on the feeding reactions of wireworms. Using sand at various percentages of saturation (the value for saturation was obtained by adding water to the sand in a measuring cylinder until free water just appeared at the surface), Lees concluded that there is a very pronounced tendency for wireworms to feed when the soil is relatively dry, the reverse being true of wireworms in wet soil. At first sight these conclusions appear to conflict, in part, with the results reported above which show that the amount of food consumed is greater in moist soil than in wet and dry soils. Lees carried out his experiments in pure sand with a grain size of 0.05–0.1 mm., but in his discussion of the results he uses the word 'soil' for such material. Thus although sand at 10 % saturation may be 'dry' relative to 80 % saturation, the suction force of the water at 10 % saturation will be relatively low and would be comparable to that of moist soil rather than 'relatively dry soil'.

Evans & Gough (1942) suggested that increase in dry weight is a better criterion of growth than increase in fresh weight. To test this idea further the dry weights of all the wireworms were determined at the end of the experiment. Fig. 9 shows the mean increase in dry weight of wireworms at the various contents of soil moisture. The shape of the curve is very similar to that for the amount of food consumed at various moisture contents and suggests a high correlation between the amount of food consumed and the increase in dry weight. Thus it appears that the maximum increase of fresh weight at 50 and 65 % soil moisture is due to an increased uptake of water and not of dry matter.

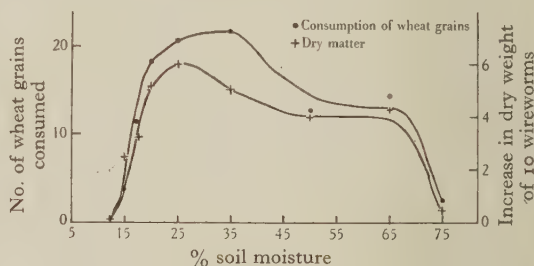


Fig. 9. The effect of varying soil-moisture content on consumption of food and increase in dry matter of wireworms.

(iv) *The value of the *pF* scale of soil moisture for expressing the soil-moisture relations of wireworms*

Schofield (1935) and Botelho da Costa (1938) have shown that the wetness of a soil can be conveniently expressed in terms of the sum of the various forces acting on the water present. The water present in a soil which is not waterlogged is at a pressure less than atmospheric pressure by an amount which it is convenient to call the suction force. The 'wetness' of the soil is expressed by the logarithm of the suction force required to dry the soil from saturation to that degree of wetness. Thus if a layer of wet soil is placed on a fine layer of silt on a filter paper in a Buchner funnel (the layer of silt allows water to pass through readily but prevents the passage of air), it is possible to maintain any desired pressure difference up to 1 atm. across the filter and measure it on a manometer. By this arrangement the moisture content of the soil at given suction force up to 1 atm. can be determined. Other methods involving the determination of the freezing-point or of the vapour-pressure depression of the soil are necessary for determining the suction forces greater than 1 atm. (Schofield, 1938; Botelho da Costa, 1938; Davidson & Schofield, 1942). The scale used to express the suction force is called the *pF* scale and is the logarithm of the height in centimetres of an equivalent water column. The maximum suction

given by a Buchner funnel does not exceed 1 atm. or 1000 cm. of water, and this suction is expressed as pF 3.

The soil-moisture conditions which are of most practical interest are not conditions of true equilibrium, and in practice there appear to be two characteristic suction forces at any one soil moisture, one when the soil is drying and the other when it is being moistened. The suction needed to withdraw water from a moist soil is, in general, greater than that against which water will enter the soil at the same moisture content. For any one soil, therefore, there are two curves relating soil moisture contents to pF , one termed the drying curve and the other the wetting curve.

Using this method of expressing the forces acting on water in the soil, Schofield (1935) and Botelho da Costa (1938) have shown that for a wide range of soils the pF at which the permanent wilting of plants occurs varies between 4.0 and 4.4, whereas the corresponding moisture contents vary from 4 to 24 %. In view of the hypothesis outlined above that the wireworm may be regarded as an osmotic system, the pF scale of expressing soil suction forces would appear to be of value, especially as it is directly related to the relative humidity of the soil atmosphere through the equation

$$pF = 6.5 + \log_{10} (2 - \log \text{R.H.})$$

(R.H. = relative humidity %). It can also be used to express the suction force of solutions of varying osmotic pressure through the equation

$$pF = 3 + \log_{10} \text{O.P.}$$

(O.P. = osmotic pressure expressed in atmospheres).

By basing the suction force on a free-energy scale it can be expressed without determining the particular mechanism, i.e. concentration of solutes, surface tension, viscosity, etc., which brings about that force.

Fig. 6 shows that a 0.33 M sucrose solution is approximately isotonic for wireworms; this solution has a pF of 3.94. Therefore it would be expected that wireworms should lose weight in soil with a pF higher than 3.9. Reference to Fig. 7 shows that starving wireworms begin to lose weight at a soil-moisture content of 19 %, and that the corresponding point of inflexion of the curve for feeding wireworms occurs at 18 %. The pF 's of these soil-moisture contents are respectively 3.8 and 4.0.

Further evidence that the loss of water in dry soil is determined by the pF is given by Fig. 10, which shows that a linear relationship exists between pF and the logarithm of the weight of the wireworms after 7 weeks.

Calculations were made to ascertain whether the loss of weight was determined by the ordinary laws of osmotic pressure, assuming that the osmotic pressure of the body fluids was chiefly determined by

crystalloids. The calculated loss of weight, in all cases, was approximately twice as great as that found. This suggests that the colloids present in the body fluids are responsible for a large fraction of the osmotic pressure.

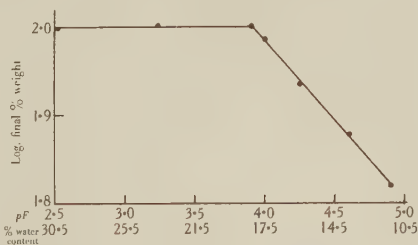


Fig. 10. The relationship between the pF and the log percentage weight of wireworms after 7 weeks.

Further critical experiments using other soils have confirmed the suggestion that the rate of loss of water is governed by the suction pressure and not by the moisture content of the soil. The moisture contents of three varying types of soil were determined at pF 5; the types of soil used and the final weights of the wireworms (expressed as percentage of original weight) after 5 weeks are shown in Table 8, which also shows the final weights of wireworms kept at a moisture content of 10.38 % in all soils.

TABLE 8. Showing that the suction force (pF) and not the moisture content of soil governs the rate of loss of water from wireworms

Type of soil	% moisture content at pF 5.0	Wt. of wireworms after 5 weeks as % original wt.
Woburn sandy	4.03	56.7
Rothamsted allotment loam	7.52	55.9
Rich potting loam	10.38	56.9
	pF at a moisture content of 10.38 %	
Rothamsted allotment loam	4.1	86.9
Woburn sandy	3.6	102.2

The final weights of wireworms kept at a constant pF but at differing soil-moisture contents are strictly comparable, but those of wireworms kept at a constant soil-moisture content and differing pF are widely different.

I am very much indebted to Dr R. K. Schofield of the Physics Department, Rothamsted Experimental Station, for his advice and help on matters concerning soil moisture.

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A further note on an unusual correlation between insect damage and crop harvested

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(With 1 Text-figure)

The unusual positive correlation between the yield of tea and damage caused by shot-hole borer beetles persisted during the third year from the pruning of the bushes even though the beetle attack fell to a low level. The diminished insect injury during the third year enabled a significant yield response to nitrogenous manures to be demonstrated. Methods of measuring beetle infestation are discussed, and the view is put forward that factors favouring growth, as reflected by crop, also favour the multiplication of the beetle.

In an earlier communication (Gadd, 1944) attention was drawn to an unusual correlation ($r = +0.927$) between the yield of tea and the damage caused by the shot-hole borer beetle (*Xyleborus fornicatus* Eichoff.). That correlation coefficient was determined at the end of the second year from the pruning of the bushes, at a time when the main attack by the beetle was considered to be completed. The experiment was continued for a further year, until 2 Sept. 1943, when the last harvest was collected and the bushes were again pruned.

Tea bushes have to be pruned at regular intervals, in this instance every third year, for two main reasons: (1) in order to maintain the bush at such a

height that the young shoots can easily be reached and (2) to prevent flowering. A common method of pruning and the one used in this experiment is to select a suitable branch near the centre of the bush and to cut it 4 in. above its base, i.e. where it joins the parent branch. This branch arose as a result of the previous pruning and its base is but a short distance below the last pruning cut. The height at which this branch is cut determines the pruning level, and all other branches are cut to that level. It will be realized that each pruning level is but 1-2 in. above the preceding level and that the prunings or pruned branches which are removed comprise the bulk of the woody matter, so far as increase in length

is concerned, produced since the last pruning. All that is left in the bush are short lengths, about 4 in. long, from which new branches will arise to give future harvests. When pruning was completed, the average height of the bushes above ground level

not a very accurate one, of the size of the bush, while the number of galleries in the prunings gives an approximate estimate of infestation by the beetles. The accuracy of this estimate will be discussed later. The galleries were recorded as open or healed, but

TABLE I. *Experimental results*

Treatment	Yield (lb.)		Broken branches		Data from 5 collections			Data from prunings		
	3rd year	Total	3rd year	Total	Broken branches	Galleries	Galleries per branch	Pruned branches	Galleries	Galleries per branch
O	226.36	658.64	1898	8249	167	303	1.814	815	1974	2.422
N	297.15	824.56	2334	10318	152	234	1.539	844	2398	2.841
P	243.13	691.95	2228	8900	166	280	1.687	697	1912	2.743
NP	283.84	784.04	2203	10611	186	299	1.607	779	2130	2.734
K	301.32	841.60	2697	11364	195	323	1.656	868	2697	3.107
NK	286.33	789.93	2187	9619	146	226	1.548	774	1958	2.530
PK	210.67	626.23	1910	8425	148	256	1.730	805	2201	2.734
NPK	270.44	753.81	2317	9528	159	268	1.686	980	2683	2.738
Total	2119.24	5970.76	17774	77014	1319	2189	1.3268	6562	17953	2.1849
Mean	264.905	746.345	2221.75	9626.75	164.87	273.625	1.6585	820.25	2244.1	2.731



Fig. 1. Graph showing weekly collections of broken branches from an area of 3.2 acres.

was 12 in., and the mean diameter of the pruned branches at the cut was 0.45 in.

After the last plucking seven bushes selected in each plot with the aid of random number tables were pruned and the numbers of cut branches and of galleries within them were counted. The number of pruned branches affords a rough measure, though

no attempt was made to estimate the actual beetle population. A summary of these records together with other relevant data are given in Table I.

The numbers of broken branches collected after each plucking from the whole experimental area (3.2 acres) are shown graphically in Fig. 1. The high value at the first collection, on 31 Jan. 1942, is due

to the fact that no previous collections had been made and it represents all the breakages that had occurred since the last pruning. The value shown at other dates represents only the breakages of that week. It will be evident from the graph that the greatest damage occurred during June and July 1942, that throughout 1943 the number of broken branches was relatively small and that there was no peak in June and July 1943 to correspond with that of 1942. The absence of a peak during 1943 may suggest that the beetles were generally less numerous that year; but that was not the case. Data from an earlier experiment (Gadd, 1941*b*, 1942), in which the increase in the number of galleries was followed, indicated that in the area under consideration shot-hole borer attack is at a low level during the third year from pruning, i.e. infestation is dependent to a large extent upon the age of the bushes from pruning.

The present observations support that conclusion in so far as there was a marked diminution of attack in the third year, if the number of broken branches affords, as I think it does, a fair measure of the beetle's incidence.

The diminution in the number of broken branches and the absence of a June-July peak were expected during 1943, the third year from pruning. During the first 2 years, when breakages were numerous and the consequent reduction in crop was material, it was impossible to demonstrate that the observed increases in yield following manuring were of statistical significance. The question naturally arose whether a decrease in insect damage during the third year would allow yield increases to reach significant values. I am indebted to my colleague Dr Eden for an examination of the yield figures, and he has supplied those relating to the apparent effect of nitrogenous manuring with the corresponding values for significance, reproduced as Table 2.

TABLE 2. *Effect of nitrogenous manuring in the presence of shot-hole borer of tea*

Year	Observed response lb./acre	Significant value lb./acre
1st	30	49
2nd	78	86
3rd	100	84
Whole cycle	208	219

Significance is achieved in the third year, but when the crop figures are taken as a whole for the complete plucking cycle the increase yield for nitrogen loses its statistical significance. A response to nitrogen was the only one expected.

Analysis of variance fails to demonstrate any significant effect from manuring on the number of broken branches occurring in the plots; yet there is a significant positive correlation between yields and

the number of breakages. Up to the end of the second year the correlation coefficient for yield and breakages (by treatments) was $+0.927$; for the third year it was found to be $+0.819$, and for the cycle as a whole $+0.928$. Increased yields are therefore accompanied by increased numbers of broken branches, i.e. by an increase in insect damage.

Despite the marked decrease in the number of breakages occurring in the third year, the treated plots on the average behaved much the same as in the previous years; the correlation coefficient for breakages in the first 2 years and in the third year has the significant value of $r = +0.794$. It is evident, therefore, that whatever early effects the manurial treatment had on the incidence of the borer beetle, as measured by broken branches, they persisted during the third year.

In the earlier paper (Gadd, 1944), possible causes of increase of insect injury accompanying increased soil fertility as shown by crop records were discussed. The evidence, though not conclusive, suggested that the number of breakages depended more upon the number of galleries made by the insects than upon any change in the fragility of the branches. The coefficient of correlation between the number of galleries per broken branch and the breakages which had occurred in the plots at that time was found to be $r = +0.655$, a value which falls short of the amount usually demanded for significance. In the third year broken branches were examined on five occasions (Collections Nos. 64, 70, 74, 78 and 82), and the number of galleries per branch for each treatment was determined. The data from all five samples were amalgamated to give one figure per treatment (Table 1). The coefficient of correlation between the number of galleries per branch and the breakages during the third year was ascertained to be $r = +0.512$ a value yet smaller than that previously determined.

A further opportunity of determining the number of galleries in the bushes was afforded by the fact that the bushes had to be pruned at the end of the experiment. As already stated, seven bushes were selected at random from each plot as samples and the branches and galleries in each sample were counted. That a high correlation ($r = +0.855$) exists between the number of galleries and the number of pruned branches is not surprising; that would exist even if all branches were equally attacked.

It might be argued that the plots which give the highest yields also have the largest bushes, yield being conditional on bush size. If that were true and if the number of branches were a fair measure of bush size for this purpose, a high r value for yield (whole cycle) and the number of pruned branches would be expected. Its value on calculation proved to be only $r = +0.028$, which indicates that if increased yields are accompanied by increased bush

size, the number of pruned branches does not afford an efficient measure of bush size. The coefficient of correlation between number of pruned branches and the number of broken branches during the plucking cycle is also low, viz. $r = +0.251$. It follows, therefore, that the relationship between yield and insect damage is in no way dependent on bush size as measured by a count of pruned branches.

The coefficients of correlation for the number of galleries in the prunings and yield for the whole cycle ($r = +0.506$) and for breakages during the cycle ($r = +0.581$) are not statistically significant, but when the galleries are expressed not as a total but as the number per pruned branch, the values become $+0.551$ and $+0.737$ respectively, the latter being statistically significant.

It seemed advisable therefore to examine further the relationship between galleries per pruned branch and the breakages which occurred during the 3-year plucking cycle. An analysis of covariance is given in Table 3, from which it will be seen that when the results from the thirty-two plots, independent of treatment and position, are examined,

the mean diameter of the pruned branches at the cuts was 0.45 in. These measurements indicate that breakages occurred above the pruning level and that the pruned branches in consequence are deficient of parts removed as broken branches. As each broken branch contained at least one gallery, a count of the galleries in the prunings at the end of the experiment falls short of the true number by a quantity varying with the number of broken branches and their gallery contents. As the bushes in high-yielding plots lose more branches, counts of galleries in their prunings are subject to greater errors than are the estimates from lower yielding plots. In all cases the error is in one direction, underestimation.

Counts of the galleries in the broken branches are still less accurate, particularly when the estimation can be made on few occasions. The coefficient of correlation between the galleries per broken branch (on five occasions between collections 64-82) and galleries per branch in the prunings is only $r = +0.328$, a value too low to be of much importance.

TABLE 3. *Analysis of covariance, galleries per pruned branch and breakages (whole cycle)*

	D/F	Sum of squares		Sum of products	r	Significance
		Galleries per pruned branch	Breaks			
Blocks	3	0.73325	296231	39.96	+0.0857	$P > 0.1$
Treatment	7	1.0289	2086247	1108.48	+0.7566	$P < 0.05$
Error	21	6.81699	2008379	2263.44	+0.6117	$P < 0.01$
Total	31	8.5791	4390857	3411.88	+0.1738	$P > 0.1$

the coefficient of correlation is small ($r = +0.174$) and not significant. When, however, the variances due to position (blocks) and to treatments are removed a significant and positive correlation becomes evident ($r = +0.612$). It seems logical to conclude therefore that the number of breakages occurring in the plots is dependent to a large extent on the number of galleries made in the branches, i.e. upon the degree of infestation by the beetle as measured by galleries per pruned branch.

Infestation by shot-hole borer in tea bushes is difficult to estimate with accuracy. Three methods have been used in this experiment, by counting (1) the broken branches, (2) the galleries, on occasions in the broken branches, and (3) the galleries in the prunings. At first sight the last method would appear to be the most reliable, but a brief consideration will be sufficient to indicate an important error. A pruned branch consists of a main stem with subsidiary branches; what have been termed broken branches are really subsidiary branches, which had they remained unbroken would have been parts of the pruned branches. The mean diameter of the broken branches measured at the break on six occasions during the third year was 0.28 in., whereas

The best measure of infestation is probably the count of broken branches. This method assumes that there is no change in the liability of branches to break after attack as a result of manurial treatment or other causes. It tacitly assumes that the number of breakages is proportionate to the number of galleries made. This assumption is difficult to prove or disprove. The analysis of covariance already discussed affords some evidence on the point, as there is, as already shown, a significant positive value for the correlation between breakages and the number of galleries per branch of the prunings.

If, therefore, we accept the number of broken branches as the best measure of infestation, it follows that manurial treatments which improve the yielding capacity of the tea bush also increase the liability of the bush to attack by the shot-hole borer beetles, and consequently to increased damage which in turn leads to a reduction in the benefit obtained from the treatment as measured by crop. Why this should be so raises a question of considerable importance, to which no very exact answer can be given at present.

So far as is known the shot-hole borer beetle of tea has few, if any, natural enemies of economic

importance. For the greater part of its life it lives within a gallery and is thereby protected against the vagaries of the weather and against many predators, as the entrance to the gallery is very small. Only during the short time which elapses from the emergence of a fertilized female from her original home till she has bored a new gallery is she exposed to any great apparent dangers. The writer is unaware of any evidence indicative of appreciable migration by the beetles, and he is of the opinion that normally the beetle migrates but a very short distance. After emergence she has to move but a short distance along the branch to find another place for entry. If this picture is correct, it seems surprising that a marked increase of infestation, as shown by gallery counts and by broken branches as in this experiment, should occur only during a relatively short period of the pruning cycle and that the infestation should subside as rapidly as it develops.

In the writer's opinion a small population of beetles remains in the frame of the bush even after the bulk of the galleries are destroyed with the prunings. That population is able to maintain itself at a very low level until conditions within the bush are favourable for rapid increase in number. When studying the life history of this beetle, Gadd (1941*a*) concluded that an appreciable number of deaths occurred amongst the young within the galleries and estimated from the data supplied a loss of 50 %. That loss may be greater or less according to the conditions prevailing within the galleries; unfavourable conditions may reduce the out-turn of beetles to a negligible quantity. During an experiment with two types of plucking it was shown that during a period of observation the number of occupied galleries in the light-plucked bushes

increased at the rate of 10.9 % per week as compared with 7.3 % per week for the hard-plucked plots, and that the rates of increase in the beetle populations of the plots were 10.0 and 7.3 % per week respectively.

These figures suggest that different types of plucking may lead to the establishment of different internal conditions in the bush affecting the rate of increase of the beetle populations. If plucking can cause such a change it would not be surprising that manurial treatments should cause equally important changes.

It must be remembered that the shot-hole borer beetle does not obtain its nutrient directly from the tea bush but indirectly from an ambrosia fungus grown on the walls of the gallery. The amount of food available for the beetles depends upon the rate of growth of the fungus. If the growth is too slow the population, particularly the very young, would be reduced; too rapid growth might be equally disastrous, as excessive growth would tend to crowd out the families. A very delicate balance must therefore exist between the growth of the ambrosia fungus and the rate of increase in the beetle population.

Although no definite explanation can be given of the positive correlation which exists between shot-hole borer infestation and soil fertility as influenced by manurial treatment and measured by crop harvested, it is the writer's opinion that such explanation must be sought amongst the interrelations between the beetle, the ambrosia fungus and the tea bush.

I am indebted to Messrs G. D. Austin and W. T. Fonseka for the collection of data upon which these observations are made.

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Trichloroacetonitrile as a fumigant

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The comparative toxicities of trichloroacetonitrile to various insects infesting stored products and houses have been determined. The toxicity is increased by the addition of 10 % or more of carbon dioxide. In the fumigation of bulk wheat trichloroacetonitrile compares favourably with ethylene oxide. Its phytocidal action on seed wheat at doses which might be used in practice is not serious and the vitamin B₁ and riboflavin contents of wheat are not affected. The possibility of fumigating dried fruit with trichloroacetonitrile has been demonstrated. Trichloroacetonitrile is highly phytocidal to growing plants.

INTRODUCTION

Hydrogen cyanide and ethylene oxide, the two best known and widely used fumigants, possess disadvantages which, to some extent, offset their high insecticidal properties. It is, therefore, eminently desirable to continue the search for a fumigant which is highly lethal to insects, but safe, or relatively safe, for use in fumigation practice.

A new fumigant named trichloroacetonitrile has been tested on a practical scale in Germany, and Peters (1940) reviews its properties and the results of practical tests carried out in dwelling houses and warehouses. Its use in Germany was described in the *Chemical Trade Journal* for 7 March 1941.

Trichloroacetonitrile seems to have many desirable properties as a fumigant. In spite of its relatively high b.p. of 85° C., it is readily vaporized. The vapour is nearly 5 times as heavy as air, and at 25° C. the saturation concentration is about 600 mg./l., that is, 20 times the concentration necessary for practical fumigation. Its low absorption by materials also facilitates airing, and houses may be reoccupied without danger a few hours after fumigation. The toxicity of trichloroacetonitrile to man is comparable to that of ethylene oxide, and its strong lachrymatory effects give warning of its presence even at concentrations of 5–10 mg./cu.m. Trichloroacetonitrile is non-inflammable and does not affect metals, polish, paints, silks and other clothing, or dried fruits but at high humidities it attacks iron and steel. Gassner (1940) found that trichloroacetonitrile under certain conditions gave rise to phosgene but stated that if directions are followed there need be no hesitation about using trichloroacetonitrile as a fumigant. Busbey *et al.* (1942) studied the toxicity of trichloroacetonitrile to the Californian red scale (*Aonidiella aurantii* Mask.) and found it to be somewhat less toxic than HCN, but much more toxic than methyl bromide. However, it was phytocidal to lemon fruits.

EXPERIMENTAL RESULTS

Preliminary tests on various insects

The results of experiments on the toxicity of trichloroacetonitrile to four common insect pests are given in Table 1 which gives the percentage mortalities obtained.

TABLE 1. *Toxicity, in terms of average percentage mortality, of trichloroacetonitrile to four insect pests*

Conc. mg./l.	<i>Calandra</i> <i>granaria</i>	<i>Tribolium</i> <i>castaneum</i>	<i>Ephestia</i> <i>kühniella</i> (larvae)	<i>Cimex</i> <i>lectularius</i>
39.1	100	100	100	100
22.2	100	100	100	100
15.7	30	100	100	100
8.0	90	69	100	100
5.8	7	34	100	59
3.3	18	8	91	11
1.2	0	0	0	0
Control	0	1	10	10

The trichloroacetonitrile was introduced under partial vacuum, by means of a small graduated pipette, into a desiccator which contained the insects in muslin bags. The bags were sealed in glass bulbs. The air in the desiccator was then brought back to normal pressure and the bulbs broken to release the insects. An exposure of 5 hr. at 20° C. was given and mortality counts made 7 days later. The usual variation in resistance among the different species of insects occurred, *Cimex lectularius* and the larvae of *Ephestia kühniella* being the least resistant species.

For comparative purposes the toxicity of the ethylene oxide and HCN to the same four insects under similar conditions are given in Table 2. The doses required for 50 and 99 % kills were estimated by the Probit method devised by Bliss.

The preparation of complete mortality curves with respect to the toxicity of trichloracetoneitrile to the above-mentioned four insects was not considered desirable at this stage, and further experiments were made to determine the resistance of other stored products insects to trichloracetoneitrile. The experimental conditions were the same as for previous tests and the results are recorded in Table 3. *Calandra granaria* was included in this series because of its high resistance and because it showed a rather large variability in the previous experiments.

From these experiments it is seen that only the larvae of *Trogoderma versicolor* were more resistant than *Calandra granaria*.

About 480 lb. of Canadian wheat were added to the bin which was then nearly two-thirds full. During the process of filling the bin five small, wire gauze cages, each containing fifty specimens of *Calandra granaria*, were placed on the vertical axis of the bin at intervals of 10.5 cm., cage 5 being placed on the bottom.

The fumigants were applied by pouring the requisite quantity into a Petri dish laid on the surface of the wheat, after which the bin was closed and left for periods of 17, 24 and 48 hr. The temperatures inside the bin during the fumigation were measured by a maximum and minimum thermometer placed on the surface of the wheat. After fumigation the insects were removed and aired for 48 hr. Mortality

TABLE 2. Comparative toxicities in mg./l. of HCN and ethylene oxide

Fumigant	% kill expected	<i>Calandra granaria</i>	<i>Tribolium castaneum</i>	<i>Ephestia kühniella</i> (larvae)	<i>Cimex lectularius</i>
HCN	50	10.2	0.19	0.13	0.11
"	99	28.3	0.54	0.33	0.33
Ethylene oxide	50	6.7	10.6	5.4	—
"	99	13.3	35.4	19.1	—

TABLE 3. Toxicity of trichloracetoneitrile to various other insects

Insect species	Av. % mortality obtained			
	(Control) 0 mg./l.	5 mg./l.	15 mg./l.	25 mg./l.
<i>Sitodrepa panicea</i>	7	92 (2)	—	—
<i>Calandra oryzae</i>	0	44 (4)	99 (4)	98 (2)
<i>Calandra granaria</i>	2	11 (2)	23 (4)	70 (4)
<i>Plinus tectus</i>	8	59 (4)	100 (2)	100 (2)
<i>Tenebrio molitor</i> (larvae)	0	15 (2)	98 (2)	97 (2)
<i>Tineola bisselliella</i> (larvae)	0	13 (2)	100 (2)	100 (2)
<i>Trogoderma versicolor</i> (larvae)	2	16 (2)	15 (4)	50 (4)
<i>Anthrenoceros australasiae</i> (larvae)	3	32 (4)	98 (4)	100 (2)

(Number of replicates in brackets.)

Grain fumigation experiments

In the fumigation of grain in bulk the efficiency of an insecticide depends to a large extent upon the ease with which it penetrates the intergranular spaces. The vapour density of trichloracetoneitrile is 4.95 (air = 1), and thus the fumigant would tend to diffuse readily downwards through a porous mass. In the experiments described below the penetration into a mass of wheat was compared with that of ethylene oxide (vapour density 1.52), which is commonly used for grain fumigation in this country.

The fumigations were carried out in a cylindrical iron bin, 74 cm. diameter and 90 cm. deep, with a capacity of about 390 l. The bin was provided with an iron cover which could be closed by hand-screws against a rubber gasket.

counts were made 5 days later. The results of the experiments are given in Table 4.

Under these conditions the limited data shown in Table 4 confirm that the efficiency of trichloracetoneitrile as a grain fumigant is substantially similar to that of ethylene oxide, and also that the losses due to absorption of fumigant by grain cannot be high. It should be noted that the experimental temperatures were low and therefore conducive to high absorption.

After ventilation for 48 hr. no trace of either fumigant was detectable by odour. This is an important fact. Residual trichloracetoneitrile would be objectional because very small amounts of vapour can be detected by odour. Additional work is necessary to determine if any decomposition occurs within the grain. Arrangements have been made for

TABLE 4. *Comparison of trichloracetoneitrile and ethylene oxide in fumigation of grain containing Calandra granaria*

Fumigant	...	Trichloracetoneitrile				Ethylene oxide		
Exposure (hr.)	...	17	24		48	24		48
Dose (mg./l.)	...	48	48	60	48	48	60	48
% mortality in cage 1		100	100	100	100	100	100	100
"	2	100	100	100	100	100	100	100
"	3	100	100	100	100	100	100	100
"	4	100	35	100	100	90	100	87
"	5	48	4	100	65	6	100	49
Control		0	0	0	0	0	0	0
Temp. °C.		11-18	15-24	16-20	12-17	15-18	6-14	11-17

this to be done and for the chemical estimation of any residues in grain.

Effect of trichloracetoneitrile on the germination of wheat

Ethylene oxide at a normal fumigation dose of 2 lb./1000 cu.ft. for 24 hr. reduces the germinating capacity of wheat (Hoyt, 1928) from 85 to 30 %. To determine the phytocidal effect of trichloracetoneitrile on wheat, small quantities of the English variety 'White Victor' were fumigated in desiccators at 20° C. The wheat was placed in a shallow layer on a wire grid supported on the shelf of the desiccator and fumigated with a measured quantity of trichloracetoneitrile introduced under partial vacuum. Exposures of 24 and 48 hr. were given.

For the germination tests twenty-five grains of wheat were taken at random from each of the fumigated and unfumigated batches of grain and sown in 200 g. of sand (+ 10 % moisture). After 6 days at 20° C. in a greenhouse, the mean percentage germination and the mean length of the shoots were determined from four replicate experiments.

The results in Table 5 show that the effect on germination of this sample of wheat was not severe. An exposure of 4 lb./1000 cu.ft. for 24 hr. only delayed growth slightly, while 3 lb./1000 cu.ft. for 48 hr. reduced germination from about 80 to about 60 %.

Effect of trichloracetoneitrile on vitamin B complex in wheat

It would be undesirable to use as a grain fumigant any chemical likely to have an adverse effect upon the vitamin B complex and although it was not

TABLE 5. *Effect of trichloracetoneitrile on germination of wheat*

Dose mg./l.	Volume of desiccator l.	Exposure hr.	Mean % germination	Mean coleoptile length mm.
59.3	5.8	24	77	19
Control	—	—	74	27
62.8	5.8	24	83	33
46.9	7.5	48	59	26
45.0	9.5	48	64	25
Control	—	—	81	33

expected that trichloracetoneitrile would cause such effects the possibility could not be ignored.

Two types of wheat were tested, namely, (1) No. 1 Manitoba, a hard wheat, defined in peace-time as the variety 'Marquis'; (2) 'White Victor', a softer variety of English wheat. Samples of 500 g. were exposed in desiccators for 48 hr. at 20° C. to a concentration of 72 mg./l. (4.5 lb./1000 cu.ft.) of trichloracetoneitrile. After airing for 24 hr. the treated and untreated samples were examined for vitamin B₁ and riboflavin. The examination furnished the data given in Table 6.

Taking the wheat in pairs there is no significant difference between A and B and between C and D. It may safely be assumed that the treatment had absolutely no effect on the vitamin B content.

Fumigation of dried fruit

To determine whether trichloracetoneitrile would be a suitable fumigant for dried fruits the opportunity was taken of fumigating four boxes of currents naturally infested with *Oryzaephilus* (*Silvanus*)

TABLE 6. *Contents of vitamin B₁ and riboflavin in fumigated and unfumigated wheat*

Wheat var.	Treatment	B ₁ I.U./g.	Riboflavin mg./100 g.
A. No. 1 Manitoba	Fumigated	1.25	140
B. "	Not fumigated	1.30	135
C. White Victor	Fumigated	1.10	120
D. "	Not fumigated	1.10	140

surinamensis L. As a check on the penetration of the fumigant through the fruit, a number of *Calandra granaria* were secreted in the fruit.

The experiments were carried out similarly to those made on wheat. The boxes of fruit were placed upright in the bin, one long side resting on two bricks. Exposures of 24 and 48 hr. were given during which the maximum and minimum temperatures in the bin were noted. A parallel experiment with ethylene oxide at a concentration of 48 mg./l. for 48 hr. was made.

A hole of 1 in. diameter was drilled through the side of each box and carried as far as the centre of the mass of currants. At the bottom of the hole was placed a tube, 2 in. long, containing a muslin bag with fifty *C. granaria*. The inner end of the tube was open and the outer end closed with muslin. After the

Toxicity of mixtures of trichloroacetonitrile and carbon dioxide

It is well known that carbon dioxide enhances the toxicity of certain fumigants, and a number of experiments were carried out to ascertain its effects on the toxicity of trichloroacetonitrile. The experiments were made in desiccators as previously described and the results given in Table 8 show that the addition of 10 % or over, by volume, of carbon dioxide increases the toxicity of the fumigant. In practice the complication of adding this amount of carbon dioxide may, however, more than outweigh the advantage.

Experiments on house flies

In experiments with *Musca domestica* L. the insects, contained in wire gauze cages, were housed

TABLE 7. Comparative effects of trichloroacetonitrile and ethylene oxide in the fumigation of dried currants

Fumigant	Dose mg./l.	Time hr.	Oryzaephilus		Calandra % dead	Temp. °C.
			No. found	% dead		
Control	—	—	29	38	1	—
Trichloroacetonitrile	60	24	127	100	98	13 -20
Trichloroacetonitrile	48	48	61	100	100	10.5-13
Ethylene oxide	48	48	22	100	100	8 -11

TABLE 8. Toxicity, in terms of percentage mortality, of mixtures of CO₂ and trichloroacetonitrile

% CO ₂ by vol.	Conc. of fumigant mg./l.	Replicates	Calandra granaria	Tribolium castaneum	Ephestia kühniella (larvae)	Cimex lectularius
80	5	2	97	100	97	100
20	5	4	18	100	99	100
13	5	2	19	98	100	100
7.5	5	1	34	70	91	100
5.0	5	1	18	85	100	94
2.5	5	1	8	76	100	58
0	5	3	6	40	95	32
80	0	3	1	3	6	10

tube and insects were dropped into position the hole was packed with currants and the hole in the box plugged with a cork.

Mortality counts on the test insects were made after 7 days. Two ½ lb. samples of currants were also taken from each box and examined for live and dead *Oryzaephilus*. The results of the tests are shown in Table 7.

The results show that trichloroacetonitrile is as effective as ethylene oxide in the fumigation of infested dried fruit. The work was limited by the amount of dried fruit available, and it was not possible to make experiments with shorter exposures. The fumigated fruit was subsequently examined by representatives of the trade who reported that no difference could be detected between treated and untreated fruit.

in desiccators and the fumigant applied as previously described. The exposure was 1 hr. at 20° C., and the results of thirty-one experiments, using nearly 2400 flies, show that a concentration of at least 4 mg./l. is required to give a total kill.

Further tests were made in a nearly empty room of 1610 cu.ft. capacity in which the fumigant was applied by an Aerograph spray gun at a pressure of 15 lb./sq.in. In each of the eight experiments all trace of the fumigant had disappeared after 10 min. airing. The results of the experiments are given in Table 9.

This treatment for the control of houseflies may not be of practical value but the fact that concentration × time factor ($c \times t$) had only to be doubled in passing from laboratory scale tests in glass apparatus to a practical scale operation confirms other obser-

TABLE 9. *Toxicity of trichloracetonitrile to Musca domestica*

Conc. mg./l.	Exposure hr.	Knock-down %	Mortality %	No. of flies used	Flies liberated immediately	Remarks
8.6	1	100	100	158	After spraying	—
6.4	1	100	100	182	Before spraying	—
4.3	1	99	92	234	After spraying	—
4.3	1	100	56	177	After spraying	Windy day
3.5	2	100	100	177	Before spraying	—
2.2	2	46	6	158	Before spraying	—
3.2	3	—	56	272	Before spraying	—
2.2	3	43	17	176	Before spraying	—

variations that high absorption of trichloracetonitrile does not occur.

If this method of fumigation could be used for the fumigation of domestic property for the control of bed bugs considerable progress towards the solution of an important problem would be made. The present most effective measure is fumigation with HCN, but this is attended by well-known difficulties such as, for instance, the danger of fumigating single rooms or flats in buildings of which the remainder is occupied. Further work is in progress.

The toxicity of trichloracetonitrile to growing plants

To determine the phytocidal action of trichloracetonitrile various potted plants were treated in a large chamber of 3280 l. capacity in which the fumi-

gant was applied by an Aerograph spray gun at a pressure of 15 lb./sq.in. The exposure was 1 hr. at 15–20° C. The results of the experiments show that a concentration of 5 mg./l. kills lettuce plants, severely scorches chrysanthemums and tomatoes, and causes slight scorching of broad beans, primulas and cinerarias. The development of trichloracetonitrile for the fumigation of plants does not appear promising.

The authors wish to thank Imperial Chemical Industries Ltd. for permission to publish these results. We are also indebted to Dr D. W. Kent Jones for the determination of vitamin B₁, and riboflavin contents of wheat and to Mr G. H. Stock for assistance in the routine work connected with the fumigation experiments.

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Reviews

A Dictionary of the Fungi. By G. C. AINSWORTH and G. R. BISBY. Pp. viii + 359. Kew: Imperial Mycological Institute. 1943. 20s.

The authors have epitomized an enormous mass of information deriving from practically the whole range of mycological and plant pathological literature and have organized it under about 10,000 headings arranged alphabetically and on lines made familiar to botanists by Willis's *Dictionary of the Flowering Plants and Ferns*. Some 7000 generic names of Eumycetes and Myxothallophyta in use to the end of 1939 are given together with their systematic position, distribution and number of species; and, in addition, there are excellent synopses often running to several pages of the chief Families, Orders and Classes of the Fungi, Bacteria and Lichens; definitions of terms used in mycology and plant pathology; the common and scientific names of numerous important fungi and of the diseases or effects they cause; pithy biographies of notable mycologists; a list of the names (and abbreviations) of authors of genera and species; lists of books, monographs and journals with their particular relevance; mycological methods, and formulae of culture media; and numerous items of interest to microbiologists, mycologists and plant pathologists. A 16-page Appendix contains the keys to the Families of Fungi from G. W. Martin's *Outline of the Fungi*, a very useful arrangement which, for many workers, is otherwise not easily available, and the book closes with 10 pages of clear outline drawings with explanations illustrating fungal structure and terminology. Throughout the text numerous references are cited giving access to fuller information.

It is difficult to see how any real improvement could be made in the general arrangement of the book or, indeed, in the detailed treatment of most of the material, although every reader will no doubt find what, to him, are sins of omission or commission. Personally, one may confess to pleasure in the decapitalization of all trivial names, a reform long accepted in zoology and long overdue in botany. What probably most readers will often wish for is much fuller information under each heading, but then the book would become an *Encyclopaedia* rather than a *Dictionary*. The book is written in Basic English which occasionally results in somewhat unfamiliar verbal usage.

The more one consults the *Dictionary* the more one realizes its comprehensiveness and critical quality, and the more one appreciates the labour and admires the scholarship and scientific acumen which have gone to its production. All microbiologists, mycologists and plant pathologists will find it invaluable and, since it is likely to become a standard work of reference, it is to be hoped that readers will give the authors the benefit of their criticisms and suggestions for consideration in future editions. The main general suggestions I would make are, firstly, that some of the older terms might be eliminated or their desuetude more clearly indicated; secondly, that the derivation of Greek and Latin terms would be a useful addition; thirdly, that the biographical notes on eminent deceased mycologists should be greatly increased in number; and, fourthly, that a serious effort should be made to reduce the cost of the book to bring it more within the reach of personal possession by students.

W. B. BRIERLEY

Bunt or Stinking Smut of Wheat. By C. S. HOLTON and F. D. HEALD. Pp. ii + 211. Minneapolis, Minn.: Burgess Publishing Co. 1941. \$3.25. (Mimeographed.)

The authors term bunt of wheat 'A World Problem', and they have abstracted and collated the world literature on this subject in a most efficient and useful way. The result is a first-class Monograph of a type greatly needed in plant pathology, which is at once a valuable source of information and a basis for further research. The subject matter is grouped into eleven chapters as follows: I, Historical introduction; II, Economic importance of bunt; III, Species distinction, spore germination and artificial culturing of the bunt fungi; IV, Host range of the bunt fungi and other species of *Tilletia* affecting wheat or other cereals; V, Factors affecting infection by the bunt fungi and their development in the host; VI, Effect of bunt on the morphology and physiology of the wheat plant; VII, Physiologic specialization in the bunt fungi; VIII, Cytology of the bunt fungi; IX, Heterothallism, hybridization, and species association in *Tilletia tritici* and *T. levis*; X, Varietal reaction and genetics of resistance to bunt; XI, Seed treatment and cultural practices for bunt control. Each chapter ends with an extensive bibliography, that to ch. XI, for example, containing 635 citations. All French citations are unaccented. The work is illustrated by good photographs and line drawings, but lacks an Index although this is, to a large extent, compensated for by detailed Tables of Contents.

W. B. BRIERLEY

The Nature and Prevention of Plant Diseases. By K. STARR CHESTER. Pp. xii + 584. Philadelphia: Blakiston Co. 1942. \$4.50.

The author states that his book has evolved and been used in mimeographed form as the basis for an elementary course to students of field crops, horticulture, soils, entomology, and related fields who have practical experience of agriculture but limited training in botany, chemistry and bacteriology, and whose interest is primarily in disease control. He therefore focuses attention on major diseases of leading crops, selecting those which illustrate the main principles of plant pathology, and emphasizes crop losses, environmental relationships, and 'epiphytological peculiarities of plant diseases': little space is given to mycological detail or to parasitism *per se*. The usual textbook arrangement is departed from, the first three-quarters of the book dealing in turn with diseases caused by rusts, smuts, Hymenomycetes, Ascomycetes, Fungi Imperfecti, Phycomycetes, bacteria, viruses, parasitic phanerogams and Algae, nematodes, and physiogenic diseases, and the remainder with methods of study, effect of environment on parasitic disease, etiology and epiphytology, and disease control by quarantine, resistance, and cultural methods. Each chapter ends with selected references, nearly all American, and there are 207 text-figures, mostly good photographs of which many are original. There is a synoptic Contents and a good Index. In detail the book is arranged clearly but, here and there, it contains ambiguous sentences or what seem to be misstatements of fact. The book has been written essentially for students in the U.S.A., but the last 100-150 pages will be useful to English students as a clear outline presentation of a somewhat neglected aspect.

W. B. BRIERLEY